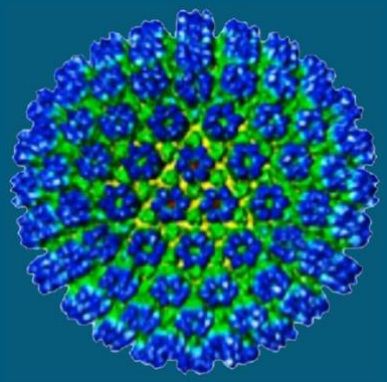
23rd International Workshop on Kaposi's Sarcoma Herpesvirus (KSHV) and Related Agents

Program and Abstracts Book



KSHV 2021

VIRTUAL MEETING

June 21-24, 2021

KSHV 2021 Virtual Meeting on Kaposi's Sarcoma Herpesvirus (KSHV) & Related Agents

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Time Zones (00:01-23:59)							
South Korea	China/ Taiwan	India	Germany	Israel/Finland/ Tanzania	EST	CST	PST
23:00	22:00	19:30	16:00	17:00	10:00	9:00	7:00
23:15	22:15	19:45	16:15	17:15	10:15	9:15	7:15
23:30	22:30	20:00	16:30	17:30	10:30	9:30	7:30
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4:30	3:30	1:00	21:30	22:30	15:30	14:30	12:30
4:45	3:45	1:15	21:45	22:45	15:45	14:45	12:45

KSHV 2021 AGENDA AT-A-GLANCE

MONDAY, JUNE 21	TUESDAY, JUNE 22	WEDNESDAY, JUNE 23	THURSDAY, JUNE 24
WELCOME	SESSION IV: VIRUS-HOST INTERACTIONS-II	POSTER II	SESSION VIII: VIRUS PATHOGENESIS AND CANCER
SESSION I: VIRUS-HOST INTERACTIONS- I	BREAK		BREAK
BREAK	SESSION V: PROCESSES OF VIRUS LATENCY	BREAK	SESSION IX: VIRUS-HOST INTERACTIONS-IV
SESSION II: CLINICAL EPIDEMIOLOGY	BREAK	SESSION VI: REPLICATION AND THERAPEUTICS	BREAK
BREAK	POSTER I	BREAK	SESSION X: THERAPEUTICS
SESSION III: IMMUNITY AND VACCINE		SESSION VII: VIRUS-HOST INTERACTIONS-III	MEETING ON NEXT MEETING, ADJOURN

KSHV 2021 Virtual Meeting on
Kaposi's Sarcoma Herpesvirus
(KSHV)
& Related Agents

23rd International Workshop on Kaposi's Sarcoma Herpesvirus & Related Agents

The KSHV 2021 Organizing Committee would like to express their sincere gratitude to the Virtual Meeting Sponsor for their generous financial support

UCDAVIS HEALTH | Department of Dermatology

KSHV 2021 Virtual Meeting on
Kaposi's Sarcoma Herpesvirus
(KSHV)
& Related Agents

Subhash C. Verma
University of Nevada, Reno
United States (Chair)

Dear Participants,

I would like to extend a sincere invitation to you all to attend the 23rd International Conference on KSHV Virtual Meeting to be held from 21st to 24th June 2021. Since before the New York meeting in 2019, we had been planning to hold the 2021 meeting in beautiful Lake Tahoe. Based upon the uncertainty of SARS-CoV-2 exposure outcome from holding an in-person gathering, meeting leadership have decided to make the 2021, an all-virtual meeting. ***On-Demand access to the oral and iPoster sessions will be available following each session, through July 10, 2021, to all registered participants.***

The Virtual Meeting format will allow broader participation and global outreach such that the numerous groundbreaking discoveries can be more quickly disseminated to the researchers and the public health partners working on KSHV and the related agents.

This conference provides a great opportunity for you to participate in the presentations and discussions on scientific advancement in KSHV and related agents, as well as network with other delegates at the conference and during virtual networking events.

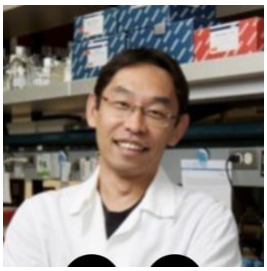
We highly encourage you to prepare and collaborate with colleagues for KSHV 2021 Virtual Meeting!

Sincerely,

Subhash Verma

Subhash Verma, University of Nevada, Reno School of Medicine
Chairperson – Local Organizing Committee

Scientific Program Committee Co-chairs



Yoshihiro Izumiya
(University of California, UC Davis)

Cyprian C. Rossetto
(University of Nevada, Reno, United States)

Dirk Dittmer
(University of North Carolina, United States)

Charles Wood
(University of Nebraska, United States)

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Zhi-Ming Zheng
National Cancer Institute, United States
Fanxiu Zhu
Florida State University, Canada
Joseph Ziegelbauer
National Institute of Health, United States

Virtual Meeting (ORAL) Presentation Information

Topic: KSHV 2021 Virtual Meeting
Time: Jun 21, 2021 07:00 AM Pacific Time (US and Canada)
Every day, until Jun 24, 2021, 4 occurrence(s)
Jun 21, 2021 07:00 AM
Jun 22, 2021 07:00 AM
Jun 23, 2021 07:00 AM
Jun 24, 2021 07:00 AM
Please download and import the following iCalendar (.ics) files to your calendar system.
Daily: https://unr.zoom.us/meeting/tZEvc-ivrjooHtXRTP-GuA5hIjIr-B_hffrS/ics?icsToken=98tyKuGtrD8rHdSUsh2GRpwMAI-gd-3ziH5BgrdlrivVTgdGQ1b-H-hrIP9dL9DW

Join Zoom Meeting
https://unr.zoom.us/j/85241701241?pwd=d0tYKzd4bk1jeFF3a28rb2pwYWVGQT09

Meeting ID: 852 4170 1241
Passcode: Provided through email

One tap mobile
+13462487799,85241701241# US (Houston)
+16699006833,85241701241# US (San Jose)

Dial by your location +1 346 248 7799 US (Houston) +1 669 900 6833 US (San Jose) +1 253 215 8782 US (Tacoma) +1 312 626 6799 US (Chicago) +1 646 558 8656 US (New York) +1 301 715 8592 US (Washington DC) Meeting ID: 852 4170 1241 Find your local number: https://unr.zoom.us/u/kbM77ODcBm Join by SIP 85241701241@zoomcrc.com	Join by H.323 162.255.37.11 (US West) 162.255.36.11 (US East) 115.114.131.7 (India Mumbai) 115.114.115.7 (India Hyderabad) 213.19.144.110 (Amsterdam Netherlands) 213.244.140.110 (Germany) 103.122.166.55 (Australia Sydney) 103.122.167.55 (Australia Melbourne) 209.9.211.110 (Hong Kong SAR) 64.211.144.160 (Brazil) 69.174.57.160 (Canada Toronto) 65.39.152.160 (Canada Vancouver) 207.226.132.110 (Japan Tokyo) 149.137.24.110 (Japan Osaka) Meeting ID: 852 4170 1241 Passcode: 57043453

Scientific Data and Recording:

Information communicated by presenters should be considered "personal communication." Please seek permission from the presenter before quoting unpublished research results or using data as a basis for further investigations. Photographs or recording (audio or visual) of data, displayed on screens, on Zoom or Poster, is forbidden.

Virtual Meeting (POSTER) Presentation Information

Guidelines:

All presenters must upload their poster to the https://www.posterpresentations.com/research/groups/unr/unr-2021-kshv.html for attendees to visit during the poster sessions on June 22nd and 23rd, 2021. The presenters may also upload an audio/video walkthrough of their poster (maximum 10 minutes). All the presenters must be present during the Live poster Q&A session, where the attendees can virtually meet the presenters and ask questions or give feedback. Live Q&A session will be held through Zoom using same link (https://unr.zoom.us/j/85241701241?pwd=d0tYKzd4bk1jeFF3a28rb2pwYWVGQT09) used for the ORAL presentation.

Due to the nature of a virtual meeting, the handling of poster materials at KSHV 2021 Online is necessarily different than an in-person meeting. When you view poster materials at KSHV 2021, remember that posters are typically works in progress. We expect poster attendees to treat virtual posters exactly as they would in-person posters and not to cite them without permission of the presenter.

SAVE THE DATES

24th International Workshop on Kaposi's Sarcoma Herpesvirus & Related Agents

June, 2022

Dar es Salaam, Tanzania

KSHV 2021 WORKSHOP OVERVIEW

Day1-June 21, 2021

WORKSHOP BEGINS
(7:00-7:30 AM)
(WELCOME NOTE)

I Virus-Host Interactions-I
(7:30-9:00 AM)
(Britt Glaunsinger & Linda van Dyk

II Clinical Epidemiology
(9:30-11:00 AM)
(Rolf Renne & Denise Whitby)

III Immunity and Vaccine
(11:30-1:00 PM)
(Blossom Damania & S. J. Gao)

Day2-June 22, 2021

IV Virus-Host Interactions-II
(7:00-8:30 AM)
(Paul Lieberman & Jennifer Toronchy)

V Processes of Virus Latency
(9:00-10:30 AM)
(Erle Robertson & Scott Wong)

Poster Session I
(11:00-1:00 PM)

Day3-June 23, 2021

Poster Session II
(7:00-9:00 AM)

VI Replication & Therapeutics
(9:30-11:00 AM)
(Dean Kedes & Zhi-Ming Zheng)

VII Virus-Host Interactions-III
(11:30-1:00 PM)
(Jae Jung & Scott Tibbetts)

Day4-June 24, 2021

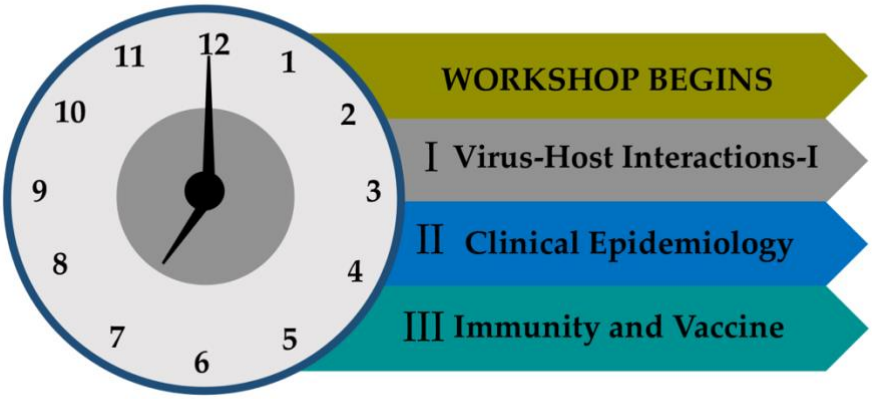
VIII Virus Pathogenesis & Cancer
(7:00-8:30 AM)
(Eva Gottwein & Pinghui Feng)

IX Virus-Host Interactions-IV
(9:00-10:30 AM)
(Thomas Schulz & Michael Lagunoff)

X Therapeutics
(11:00-12:30 PM)
(Ethel Cesarman & Laurie Krug)

WORKSHOP ENDS
(12:30-1:00 PM)
(CLOSING NOTE)

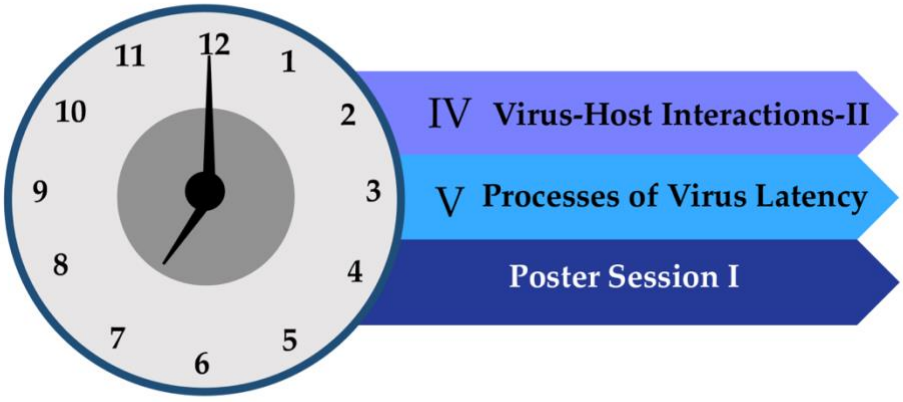
Day 1
Monday-June 21, 2021



KSHV 2021 WORKSHOP SCHEDULE		
Day 1 (Monday June 21)		
07:00 - 07:30	**Welcome**	
07:30 - 09:00	**Virus-Host Interactions-Part I**	**Session I**
Moderators: Britt Glaunsinger,University of California, Berkeley/HHMI, United States and **Linda van Dyk**, University of Colorado, Denver, United States		
07:30	Single-cell analysis of Kaposi's sarcoma herpesvirus infection in three-dimensional air-liquid interface culture model **Kyle L. Jung**, Un Yung Choi, Angela Park, Jae U Jung	1.1
07:45	Polycomb group proteins prevent aberrant transcription and episome clearance of KSHV **Weissmann Simon**, Ziegler Marion, Guenther Thomas, Grundhoff Adam	1.2
08:00	Redefining De Novo Gammaherpesvirus Infection Through High-Dimensional, Single-Cell Analysis of Virus and Host Jennifer N. Berger, Bridget Sanford, Abigail K. Kimball, Lauren M. Oko, Rachael E. Kaspar, Brian F. Niemeyer, Kenneth L. Jones, Linda F. van Dyk, **Eric T. Clambey**	1.3
08:15	Identification of KSHV episome docking sites on host chromosomes and regulation of active KSHV episome maintenance **Ashish Kumar**, Yuanzhi Lyu, Vladimir Majerciak, Michelle Salemi, Ryan R. Davis, Clifford G. Tepper, Chie Izumiya, Mel Campbell, Zhi-Ming Zheng, & Yoshihiro Izumiya	1.4
08:30	Exhaustive Functional Tiling of the KSHV Genome Identifies Essential Domains in Proteins Required for Late Gene Transcription **David W Morgens**, Divya Nandakumar, Allison L Didychuk, Britt Glaunsinger	1.5
08:45	Highly Multiplexed Tissue Imaging of Nucleic Acids and Proteins Resolves the Tissue Immune Organization During Viral Infections **Sizun Jiang**, Chi Ngai Chan, Xavier Rovira-Clavé, Han Chen, Yunhao Bai, Bokai Zhu, Erin McCaffrey, Noah Greenwald, Candace Liu, Jason L Weirather, John Paul Oliveria, Marc Bosse, Janos Demeter, Darci Philips, Nilanjan Murkherjee, Yury Golstev, David McIlwain, Michael Angelo, Jacob D. Estes, Garry P. Nolan	1.6
09:00 - 09:30	**Break**	
09:30 - 11:00	**Clinical Epidemiology**	**Session II**
Moderators: Rolf Renne, University of Florida, United States and **Denise Whitby**, National Cancer Institute, United States		
09:30	Cytokines, Chemokines, and HIV Viral Load are Associated with Recurrence of HIV-associated Kaposi's Sarcoma **Owen Ngalamika,** Marie Claire Mukasine, Musonda Kawimbe, Faheema Vally	2.1
09:45	The contribution of KSHV and/or EBV co-infection to COVID-19 severity and outcome in South African patients **Melissa J Blumenthal**, Catherine Riou, Robert J Wilkinson, Georgia Schäfer	2.2
10:00	International epidemiology of KSHV in HIV-1 infected individuals on cART **Nazzarena Labo**, Wendell Miley, Thomas B Campbell, Denise Whitby	2.3
10:15	SERIAL PROFILING OF TUMOR-INFILTRATING LYMPHOCYTES IN KAPOSI SARCOMA **Edus H. Warren**, Andrea M.H. Towlerton, Shashidhar Ravishankar, David G. Coffey, Peter Mooka, James Kafeero, Vésteinn Thorsson, Warren T. Phipps	2.4
10:30	Whole-genome sequencing of KSHV from patient samples: Evidence for a distinctly African Lineage **Razia Moorad**, Angelica Juarez, Justin T. Landis, Avery Cheves, Dirk P. Dittmer	2.5
10:45	Immune and Virus-specific Responses in Participants with Primary Effusion Lymphoma Receiving Lenalidomide, Dose-adjusted EPOCH, and Rituximab (EPOCH-R2) **Kathryn Lurain,** Romin Roshan, Ramya Ramaswami, Ralph Mangusan, Anaida Widell, Irene Ekwede, Joseph Ziegelbauer, Thomas S. Uldrick, Denise Whitby, Robert Yarchoan, and Laurie T. Krug	2.6
11:00 - 11:30	**Break**	
11:30 - 1:00	**Immunity and Vaccine**	**Session III**
Moderators: Blossom Damania, University of North Carolina at Chapel Hill, United States, and **S. J. Gao**, University of Pittsburg, United States		

11:30	ADAR1 Facilitates KSHV Lytic Reactivation by Modulating the RLR-Dependent Signaling pathway **Huirong Zhang**, Guoxin Ni, Blossom Damania	3.1
11:45	N6-methyladenosine (m6A) Mediate Innate Immune Response During Bacteria and Viral Infections **Jian Feng**, Teng Zhang, Océane Sorel, Wen Meng, Xinquan Zhang, Yidong Chen, Yufei Huang and Shou-jiang Gao	3.2
12:00	Viral miRNA target identification reveals a surprising role for EWSR1 in germinal center B cell responses **Yiping Wang**, Emily R. Feldman, Whitney L. Bullard, Scott A. Tibbetts	3.3
12:15	Vaccination with a Replication-Dead Gammaherpesvirus Protects against Wild-Type Virus Replication, Reactivation, and Disease in Mice Wesley A. Bland, Shana Owens, Kyle McEvoy, Chad H. Hogan, Luciarita Boccuzzi, Varvara Kirillov, Camille Khairallah, Brian Sheridan, J. Craig Forrest, **Laurie T. Krug**	3.4
12:30	Investigating the activation of host caspases during KSHV lytic infection as a mechanism of immune evasion **Rachel C. Lent**, Tate Tabtieng, Richard Lavin, Marta M. Gaglia	3.5
12:45	Kaposi's sarcoma-associated herpesvirus (KSHV) specific IFN-□ T cell responses in HIV seronegative individuals from rural Uganda **Angela Nalwoga**, Romin Roshan, Kyle Moore, Vickie Marshall, Wendell Miley, Nazzarena Labo, Marjorie Nakibuule, Stephen Cose, Robert Newton, Rosemary Rochford and Denise Whitby	3.6

Day 2
Tuesday-June 22, 2021

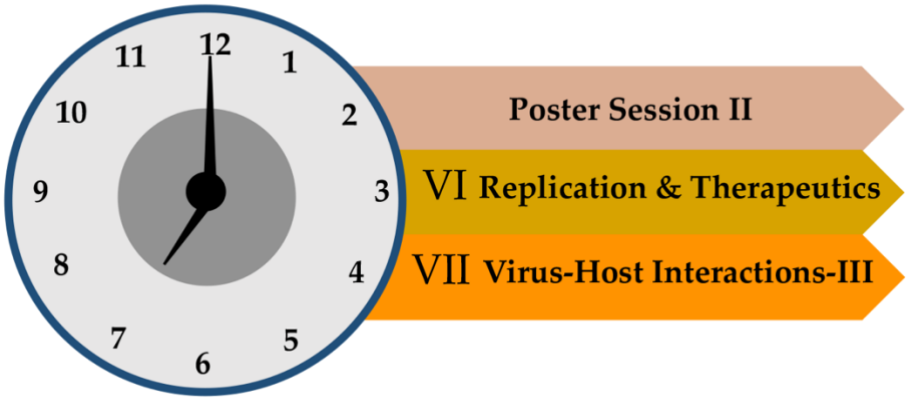


KSHV 2021 WORKSHOP SCHEDULE		
Day 2 (Tuesday June 22)		
07:00 - 08:30	**Virus-Host Interactions-Part II**	**Session IV**
Moderators: Paul Lieberman, Wistar Institute, United States, and **Jennifer Totonchy**,Chapman University, United States		
07:00	NDRG1 facilitates lytic replication of Kaposi's sarcoma-associated herpesvirus by maintaining the stability of the KSHV helicase **Lianghui Dong**, Jiazhen Dong, Min Xiang, Ping Lei, Zixian Li, Fang Zhang, Xiaoyi Sun, Danping Niu, Lei Bai, Ke Lan	4.1
07:15	Kaposi's sarcoma-associated herpesvirus processivity factor (PF-8) promotes PARP1 degradation and lytic replication by recruiting cellular E3 ubiquitin ligase CHFR Woo-Chang Chung, Seungrae Lee, Yejin Kim, and **Moon Jung Song**	4.2
07:30	A Cytoplasmic Histone Deacetylase Regulates Reactivation of Kaposi's Sarcoma-associated Herpesvirus **Helena Mello** and David M. Lukac	4.3
07:45	An in-depth analysis of the subcellular localization of the KicGAS (KSHV inhibitor of cGAS) protein in KSHV **Siming Ma**, Noah Benscher, John Henriques, Linhui Li, Jianjun Wu, Hongyu Zhang, Michael Wrobel, Arianna Broad, Debipreeta Bhowmik, Ruiming Hu, Hryhotiy Zhoba, Fanxiu Zhu	4.4
08:00	Interferon-induced transmembrane proteins inhibit infection by the gamma2-herpesviruses KSHV and RRV in a cell type-specific manner Bojan F. Hörnich, Anna K. Großkopf, Candice J. Costa, Sarah Schlagowski, **Alexander Hahn**	4.5
08:15	Establishing the role of plasma cells in early KSHV infection in B lymphocytes Romina Nabiee, Nedaa Alomari, Feryal Aalam, **Jennifer E. Totonchy**	4.6
08:30 - 09:00	**Break**	
09:00 - 10:30	**Processes of Viral Latency**	**Session V**
Moderators: Erle Robertson, University of Pennsylvania, United States, **Scott Wong**, Oregon Health Science University, United States		
09:00	The Role of Subnuclear Compartments in KSHV latency establishment **Romina Vargas-Ayala**, Thomas Günther, Adam Grundhoff	5.1
09:15	KSHV-encoded vCyclin can modulate HIF1α levels to promote DNA replication in hypoxia **Rajnish Kumar Singh**, Yonggang Pei, Dipayan Bose, Zachary L Lamplugh, Kunfeng Sun, Yan Yuan, Paul Lieberman, Jianxin You, and Erle S Robertson	5.2
09:30	A KSHV LANA Repeat Element Demonstrates Selective Binding and Mediates Viral Persistence Franceline Juillard, Marta Pires de Miranda, Shijun Li, Aura Franco, Andre Seixas, Bing Liu, Angel L. Alvarez, Min Tan, Agnieszka Szymula, J. Pedro Sima, **Kenneth M. Kaye**	5.3
09:45	Identification of Nitrotyrosine and LANA Nuclear Bodies in KS Tumors Olga Vladimirova, Alessandra De Leo, Zhong Deng, Andreas Wiedmer, James Hayden, Salum J. Lidenge, Andrew V. Kossenkov, For Yue Tso, Owen Ngalamika, John Ngowi Richards, Julius Mwaiselage, John T. West, and Charles Wood, and **Paul M. Lieberman**	5.4
10:00	A panel of KSHV recombinant viruses targeting the polycistronic kaposin locus for precise analysis of individual protein function **Mariel Kleer**, Grant MacNeil, Eric S. Pringle, Julie Ryu, Craig McCormick and Jennifer A. Corcoran	5.5
10:15	RYBP is Recruited to the KSHV Genome in PRC1-independent Manner and Represses Lytic Genes During Primary Infection See-Chi Lee1, Nenavath Gopal Naik1, and **Zsolt Toth**	5.6
10:30 - 11:00	**Break**	
11:00 - 1:00	**Poster Session I**	
Anti-inflammatory agent dexamethasone suppresses KSHV-mediated oncogenesis by inducing interleukin-1 receptor antagonist **Luping Chen**, Shou-Jiang Gao		P1.1
The cellular Notch1 Protein Promotes KSHV reactivation in an Rta-dependent manner.		P1.2

This Poster has been moved to Poster Session II (P2.27)	
Incoming KSHV Factors Reprogram Oral Epithelial Cells during Infection Natalie Atyeo, Seung Jin Jang, Min Chae, Aria Sharma, Katherine Glickman, Thomas Hong Nguyen, Zsolt Toth, **Bernadett Papp**	P1.3
Uracil DNA Glycosylase of Gammaherpesvirus binds abasic site with high affinity and supports high-fidelity uracil repair with distinct motifs **Yunxiang Mu**, Monika Anna Zelazowska, Zaowen Chen, Joshua Plummer, Laurie Krug and Kevin M. McBride	P1.4
Role of Kaposi Sarcoma-associated Herpesvirus Glycoproteins in Viral Infection **Murali Muniraju**, Lorraine Z Mutsvunguma, Gabriela M Escalante, Esther Rodriguez, Javier Gordon Ogembo	P1.5
Characterizing Expression and Regulation of Human and Viral Circular RNAs **Takanobu Tagawa**, Daniel Oh, Jerico Santos, Vishal Koparde, Kathryn Lurain, Ramya Ramaswami, Thomas Uldrick, Robert Yarchoan, and Joseph Ziegelbauer	P1.6
Phase I Study of Lenalidomide Combined with Dose-adjusted EPOCH and Rituximab (EPOCH-R2) in Primary Effusion Lymphoma in Participants with or without HIV (NCT02911142) **Kathryn Lurain**, Ramya Ramaswami, Anaida Widell, Irene Ekwede, Ralph Mangusan, Jomy George, Elaine S. Jaffe, Stefania Pittaluga, Maryalice Stetler-Stevenson, Mark Roth, Constance M. Yuan, Hao-Wei Wang, Vickie Ann Marshall, Denise Whitby, Thomas S. Uldrick, and Robert Yarchoan	P1.7
IKKalpha-Mediated Non-Canonical NF-kappaB Signaling is Required to Support Murine Gammaherpesvirus 68 Latency In Vivo Brandon Cieniewicz, Varvara Kirillov, Darby G. Oldenburg, Julie A. Bettke, **Laurie T. Krug**	P1.8
Regulation of LANA expression through the interaction of nucleolin with g-quadruplexes in the mRNA **Andrew R. Zareie**, Prerna Dabral, Subhash C. Verma	P1.9
A Pentameric Protein Ring with Novel Architecture Is Required for Herpesviral Packaging **Allison Didychuk**, Stephanie Gates, Matthew Gardner, Lisa Strong, Andreas Martin, Britt Glaunsinger	P1.10
Pomalidomide-Mediated Caspase Cleavage of K3 and K5 Prevents MHC-I Downregulation in Lytic Primary Effusion Lymphoma Cells **David A. Davis,** Alexandria Stream, Hannah K Jaeger, Prabha Shrestha, Hong S. Choi, Robert Yarchoan	P1.11
Targeting MYC Transcription with Small Peptide Derived from KSHV Transactivator Michiko Shimoda, Yuanzhi Lyu, Kan-Hsing Wang, Ashish Kumar, Ryan R. Davis, Chanikarn Chantarasrivong, Chie Izumiya, Clifford G. Tepper, Gustavo Barisone, and **Yoshihiro Izumiya**	P1.12
Regulation of Immune Surface Molecules by CDK4/6 Inhibitors in Gammaherpesvirus-infected Tumor Cells **Wu Y,** Shrestha P, Heape NM, and Yarchoan R	P1.13
Characterizing Cellular Mutations in Kaposi Sarcoma Tumors **Warren Phipps**, Bhavneet Bhinder, Andrea Towlerton, Peter Mooka, James Kafeero, Olivier Elemento, Ethel Cesarman	P1.14
Inhibition of the eIF4E-kinase MNK as a Strategy for Controlling Kaposi's Sarcoma-Associated Herpesvirus Replication **Catya Faeldonea,** Kevin Brackett, Carolina Arias	P1.15
Prox1-induced Lytic replication of Kaposi sarcoma herpes virus promotes Kaposi sarcoma development Joshua Jin Choi, Dongwon Choi, Eunkyung Park, Young Jin Seong, Luping Zhao, Ji-Eun Irene Yum, Il-Taeg Cho, and Young-Kwon Hong	P1.16
ORF45 is a novel inhibitor of p53-signalling encoded by Kaposi Sarcoma-associated Herpesvirus Dina Alzhanova, James O. Meyo, Angelica Juarez, and Dirk P. Dittmer	P1.17
A KSHV microRNA Downregulates Anti-Angiogenic Cellular lncRNA MEG3 in Endothelial Cells **Lauren A. Gay**, Daniel Stribling, Melody Baddoo, Erik Flemington, Nate Ungerleider, & Rolf Renne	P1.18
High KSHV seroprevalence among MSM Persons with HIV (PWH) in an urban safety-net health center in the southern United States; evidence of racial/ethnic disparities **Sheena Knights**, Susana Lazarte Maverick Salyards, Radhika Kainthla, Nazzarena Labo, Wendell Miley, Vickie Marshall, Denise Whitby, Elizabeth Chiao, Ank Nijhawan	P1.19
Evidence for multiple subpopulations of gamma herpesvirus-latently infected cells	P1.20

Justin Landis, Ryan Tuck, Yue Pan, Carson N. Mosso, Brent Eason, Razia Moorad, Steven Marron and Dirk P. Dittmer	
Systematic Analysis of KSHV Genome Sequences from a Cameroonian Kaposi's sarcoma Case-Control Study finds no Association with Disease Risk **Vickie A Marshall**, Nicholas C Fisher, Charles Goodman, Elena M Cornejo Castro, Sirish Khanal, Benjamin Holdridge, Abbey Thorpe, Nazzarena Labo, Isabella Liu, Kristen Stolka, Paul N'Dom, Jennifer J. Hemingway-Foday, Mahamat Abassora, Jennifer S. Smith, Neneh Sallah, Anne Palser, Paul Kellam, Brandon F Keele, and Denise Whitby	P1.21
WT1 Tumor Antigen is Overexpressed in Kaposi Sarcoma and Is Regulated by KSHV vFLIP **Ayana Morales**, Caitlyn Genovese, Matthew Bott, Julio Alvarez, Sung Soo Mun, Jennifer Totonchy, Archana Gautam, Jesus Delgado de la Mora, Stephanie Chang, Maite Ibáñez de Garayo, Dagmar Wirth, Marcelo Horenstein, Tao Dao, David A. Scheinberg, Paul G. Rubinstein, Aggrey Semeere, Jeffrey Martin, Margaret Borok, Thomas B. Campbell, Susan E. Krown, Ethel Cesarman	P1.22
Elucidation of Biomarkers from Kaposi's Sarcoma Transcriptomics and Validation in Tumor Tissues Sara R. Privatt, Charles Wood, John T. West	P1.23
Gene Expression Patterns in Skin and Gastrointestinal Kaposi Sarcoma Lesions Ramya Ramaswami, Takanobu Tagawa, Vishal Koparde, Kathryn Lurain, Ralph Mangusan, Guruswamy Mahesh, Anna Serquina, Anaida Widell, Irene Ekwede, Robert Yarchoan, Joseph Ziegelbauer	P1.24
Maternal Kaposi's sarcoma herpesvirus (KSHV) Antibodies and Risk of KSHV Seroconversion in a Cohort of Kenyan Children **Katherine R Sabourin**, Conner L Jackson, Wendell Miley, Nazzarena Labo, Robert Newton, Denise Whitby, Rosemary R Rochford	P1.25
Daratumumab as a Potential Treatment for Primary Effusion Lymphoma **Prabha Shrestha,** David A. Davis, Robert Yarchoan	P1.26
Kaposi's sarcoma-associated herpesvirus transcription regulation mechanism through interactions with non-coding (nc) 7SK RNA **Shannon Harger,** and Cyprian C. Rossetto	P1.27
Epigenetic reprograming of KSHV genome during hypoxic reactivation Rajnish Kumar Singh, **Dipayan Bose**, Erle S Robertson	P1.28

Day 3
Wednesday-June 23, 2021

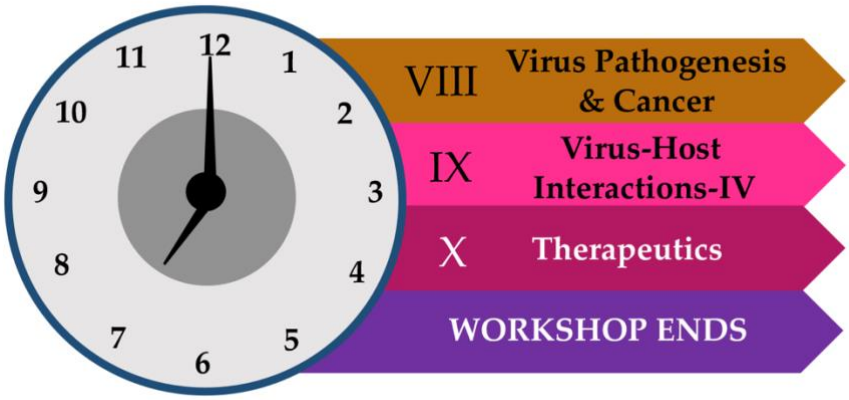


KSHV 2021 WORKSHOP SCHEDULE	
Day 3 (Wednesday June 23)	
07:00 - 09:00 **Poster Session II**	
This Poster has been removed	P2.1
CRISPR-PITA: A CRISPR/dCas9 Based Assay to Determine Recruitment Relations Between Proteins Ido Lavi, **Supriya Bhattacharya**, Ola Orgil, Nir Avital, Guy Journo, Vyacheslav Gurevich and Meir Shamay	P2.2
Phosphatase PP2A promotes dephosphorylation of RTA to inhibit KSHV lytic reactivation **Lei Bai**, Lianghui Dong, Jiazhen Dong, Ke Lan	P2.3
Decreased Virion Production of Kaposi's Sarcoma-associated Herpesvirus (KSHV) in HMGB1 Knockout Host Cell through the Compromised Viral Gene Expressions. **Su-kyung Kang**, Yun Hee Kang, Myung-Shin Lee	P2.4
Transcriptom-wide siRNA screening identifies long noncoding RNA as novel epigenetic regulator for oncogenic demethylase KDM4A **Wan-Shan Yang**, Wayne W. Yeh, Mel Campbell, Lung Chang, Pei-Ching Chang	P2.5
Comparative Efficacy of Routine Histology, IHC, and PCR Diagnostics for KS in Africa John Ngowi Richard, Julius Mwaisielage, Fred Okuku, Diana Basemera, Lazarus Okoche, Constance Namirembe, Salum Lidenge, Susan Nabadda, Andrea Towlerton, Charles Wood, John West, Warren Phipps	P2.6
Characterization of the role of murine 2'-5'-oligoadenylate synthetase-like protein 2 (mOASL2) in murine gammaherpesvirus-68 (MHV68) infection **Viktoria Vögele**, Markus Stempel, Jonas Käsbach, Marco van Ham, Lothar Jänsch, Melanie M. Brinkmann	P2.7
Recruitment of phospholipase Cg1 to the non-structural membrane protein pK15 of Kaposi Sarcoma-associated herpesvirus promotes its Src-dependent phosphorylation Naira Samarina, George Ssebyatika, Tanvi Tikla, Ja-Yun Waldmann, Vittoria Nanna, Michelangelo Marasco, Teresa Carlomagno, Thomas Krey, Thomas F. Schulz	P2.8
Iatrogenic Kaposi's sarcoma after immunosuppressive therapy: a retrospective study **L. Mezni**, F. Elhadadi, L. Benzekri, K. Senouci	P2.9
Multiplex immunohisochemistry reveals PROX1 and SOX18 co-expression with KSHV latent and lytic markers in KS biopsies **Gramolelli S.**, Elbasani E., Tuohinto K., Bower M., Pellinen T., Ojala P.M.	P2.10
The cellular DNA damage response proteins SMARCAL1 and RPA are recruited by KSHV LANA and impact viral lytic replication **Anika Freise,** Tugce Kaman, Katharina Königsfeld, David Nivia, Thomas F. Schulz	P2.11
Epidemiological Profile and Clinical Features of Kaposi Sarcoma in Rabat (Morocco). **Farah El Hadadi**, Line Mezni, Mariame Meziane, Laila Benzekri, Karima Senouci	P2.12
KSHV Uses Viral IL-6 to Exploit Monocyte Inflammatory Response **Michiko Shimoda**, Kang-Hsin Wang, Kenichi Nakajima, and Yoshihiro Izumiya	P2.13
Image-Based Analysis of Latent KSHV DNA Replication Using Engineered KSHV Sara Guevara-Plunkett, Frank Chuang, Yoshihiro Izumiya, Ken-ichi Nakajama	P2.14
Development of a concomitant surface, intracellular and RNA flow cytometry assay for detecting and phenotyping KSHV-infected cells **Elena M Cornejo Castro**, Mac Trubey, James Thomas, Cathi Pyle, Denise Whitby	P2.15
The role of histamine-related signaling in promoting viral lytic reactivation and oncogenesis **Jungang Chen**, Lu Dai, Craig Forrest, Steven Post, Zhiqiang Qin	P2.16
CRISPR Interference Efficiently Silences Latent and Lytic Viral Genes in Kaposi's Sarcoma-Associated Herpesvirus-Infected Cells **Kevin Brackett,** Ameera Mungale, Mary Lopez-Isidro, Duncan Proctor, Guillermo Najarro, Carolina Arias	P2.17
Influence of Cytokine Signaling on KSHV Transmission **Nedaa Alomari** and Jennifer Totonchy	P2.18
The Indian perspective of KSHV associated malignancy with a view of a potential natural therapeutic application **Piyanki Das**, Tathagata Choudhuri	P2.19

vIRF-1 interacts with the autophagy-competent mitochondrial translational elongation factor TUFM to inhibit caspase-8-mediated apoptosis **Chang-Yong Choi**, and Young Bong Choi		P2.20
GRWD1-WDR5-MLL2 Epigenetic Complex Mediates H3K4me3 Marks and Is Essential for KSHV-Induced Cellular Transformation **Shan Wei**[1], Lifan Liang, Xinghua Lu, Songjian Lu, Shou-Jiang Gao		P2.21
Dynamic changes in antibody repertoire of MHV68-infected B cells **Monika A. Zelazowska**, Joshua B. Plummer, Qiwen Dong, Yunxiang Mu, Laurie T. Krug, Kevin M. McBride		P2.22
The Portal Vertex of KSHV Promotes Docking of Capsids at the Nuclear Pores **Daniela (Dana) Dünn-Kittenplon**, Asaf Ashkenazy-Titelman, Inna Kalt, Jean-Paul (Moshe) Lellouche, Yaron Shav-Tal and Ronit Sarid		P2.23
Protein-RNA interactome analysis reveals wide association of KSHV ORF57 with host non-coding RNAs and polysomes **Beatriz Alvarado-Hernandez**, Yanping Ma, Nishi Sharma, Vladimir Majerciak, Maggie Cam, Alexei Lobanov, Jun Zhu and Zhi-Ming Zheng		P2.24
African Endemic and Epidemic Kaposi sarcoma in Tanzania: A comparative study in a cohort of 313 patients **Chacha J. Mwita**, Richard Ngaya, Salum J. Lidenge, For Yue Tso, John R. Ngowi, Danielle M. Shea, Gleb Haynatzki, John T. West, Charles Wood, and Julius Mwaiselage		P2.25
Pomalidomide and liposomal doxorubicin for Kaposi sarcoma +/- other KSHV diseases **Ramya Ramaswami**, Kathryn Lurain, Anaida Widell, Priscila Goncalves, Irene Ekwede, Ralph Mangusan, Jomy George, Seth Steinberg, Denise Whitby, Thomas S. Uldrick, Robert Yarchoan		P2.26
The cellular Notch1 Protein Promotes KSHV reactivation in an Rta-dependent manner Jennifer DeCotiis-Mauro, **Sun M. Han**, Helena Mello, Corey Goyeneche, Giuseppina Marchesini-Tovar, Lianhua Jin, and David M. Lukac		P2.27
09:00 - 09:30	**Break**	
09:30 - 11:00	**Replication and Therapeutics**	**Session VI**
Moderators: **Dean Kedes**, University of Virginia, United States and **Zhi-Ming Zheng**, National Cancer Institute, United States		
09:30	DNA processing by the bifunctional SOX nuclease plays a key role in KSHV genome replication and gene expression Ella Hartenian, **Aaron S. Mendez**, Allison L. Didychuk, Britt A. Glaunsinger	6.1
09:45	Glycoprotein M is an Essential Factor of Rhesus Macaque Rhadinovirus For Viral Egress **Gavin Golas** and Scott Wong	6.2
10:00	Genome-wide regulation of KSHV RNA splicing by RNA-binding protein ORF57 **Vladimir Majerciak**, Alexei Lobanov, Maggie Cam, and Zhi-Ming Zheng	6.3
10:15	The Expression and Nuclear Retention Element (ENE) of Polyadenylated Nuclear (PAN) RNA is not required for productive lytic replication of KSHV **Isaura Vanessa Gutierre,** Jacob Dayton, Shannon Harger, Cyprian C. Rossetto	6.4
10:30	Cellular kinases modulate KSHV latency maintenance **Annabel Olson**, Thomas Bello, Michael Lagunoff, Taran Gujral, Adam Geballe	6.5
10:45	Dual gRNA-directed CRISPR/Cas9 Targeting of ORF73 Eradicates KSHV Genomes and Virion Production **Matthew S. Loftus** and Dean H. Kedes	6.6
11:00 - 11:30	**Break**	
11:30 - 1:00	**Virus-Host Interactions Part-III**	**Session VII**
Moderators: **Jae Jung**, Cleveland Clinic Lerner College of Medicine, United States, and **Scott Tibbetts**, University of Florida, United States		
11:30	Kaposi's Sarcoma-Associated Herpesvirus-Encoded circRNAs are Expressed in Infected Tumor Tissues and Are Incorporated into Virions **Bizunesh Abere**, Jinghui Li, Hongzhao Zhou, Tuna Toptan, Patrick S Moore, Yuan Chang	7.1
11:45	Functional Analysis of vIRF4 Circular RNA in Kaposi's Sarcoma-Associated Herpesvirus **Soleil Torres,** Vaibhav Jain, Lauren Gay, Daniel Stribling, Melody Baddoo, Erik Flemington, Scott Tibbetts, & Rolf Renne	7.2

12:00	Kaposi's sarcoma-associated herpesvirus induces spermidine and eIF5A hypusination for viral persistency via translation control of LANA **Un Yung Choi**, Jae Jin Lee, Kyle L. Jung, Shin-Ae Lee, Hye-Ra Lee, Hyungjin Eoh, Jae U Jung	7.3
12:15	Polyamine biosynthesis and eIF5A hypusination are modulated by the DNA tumor virus KSHV and promote KSHV viral infection. **Guillaume N. Fiches**, Ayan Biswas, Dawei Zhou, Weili Kong, Maxime Jean, Netty G. Santoso, Jian Zhu	7.4
12:30	The m6A epitranscriptomic landscape of polyadenylated nuclear (PAN) RNA Sarah Elizabeth Martin, Huachen Gan, **Joanna Sztuba-Solinska**	7.5
12:45	The Host Strikes Back: C19ORF66 restricts KSHV Lytic reactivation by Inducing KSHV Viral RNA Decay **William Rodriguez**, Timothy Mehrmann, Mandy Muller	7.6

Day 4
Thursday-June 24, 2021



KSHV 2021 WORKSHOP SCHEDULE		
Day 4 (Thursday June 24)		
07:00 - 08:30	**Virus Pathogenesis and Cancer**	**Session VIII**
Moderators: **Pinghui Feng**, University of Southern California, United States and **Eva Gottwein**, Northwestern University, United States		
07:00	A Gammaherpesviral Tegument Protein Sequesters SMC Proteins and Inhibits the DNA Damage Response Florian Full, Doris Jungnickl, Brigitte Scholz, Anna Großkopf[1], Alexander S. Hahn[1], Alexandra Herrmann, **Armin Ensser**	8.1
07:15	The Central Role of KSHV in The Pathogenesis of African Endemic and Epidemic Kaposi's Sarcoma **Salum J. Lidenge**, Andrew V. Kossenkov, For Yue Tso, Jayamanna Wickramasinghe, Sara Privatt, Owen Ngalamika, John R. Ngowi, Julius Mwaiselage, Paul M. Lieberman, John T. West and Charles Wood	8.2
07:30	KSHV lytic replication plays a vital role in virus-induced lymphomagenesis during EBV co-infection **Rieble Lisa**, Caduff Nicole, Jung Jae J., Grundhoff Adam, Münz Christian	8.3
07:45	Cellular FLIP Protects KSHV-Infected Lymphoma Cells from TRAIL-Independent TRAIL Receptor 1-Mediated Death Signaling **Neil Kuehnle**, Mark Manzano, Eva Gottwein	8.4
08:00	AS1411 aptamer displays potent therapeutic potential in Kaposi's sarcoma-associated virus (KSHV)-associated Primary effusion lymphoma Miroslava Repak, Olivia Poworzek, Kumari Asha and **Neelam Sharma-Walia**	8.5
08:15	Viral glutamine amidotransferase (vGAT) activates phosphoribosyl-formylglycinamidine synthetase (PFAS) to fuel de novo purine synthesis and tumorigenesis **Ting-Yu Wang,** Xiaoxi Lin, Junhua Li, Simin Xu, and Pinghui Feng	8.6
08:30 – 09:00	**Break**	
09:00 - 10:30	**Virus-Host Interactions-Part IV**	**Session IX**
Moderators: **Michael Lagunoff,** University of Washington, United States and **Thomas Schulz,** Institute of Virology, Hannover, Germany		
09:00	Exosome-encased Nucleic Acids Scaffold Chemotherapeutic Agents for Targeted Tumor Delivery **Ryan. P. McNamara**, Anthony B. Eason, Yijun Zhou, Rachele Bigi, Jack D. Griffith, Lindsey M. Costantini, Michelle A. Rudek, Nicole M. Anders, Blossom A. Damania, and Dirk P. Dittmer	9.1
09:15	Iron Metabolism and Ferroptosis Play a Role in Kaposi Sarcoma Herpesvirus Pathogenesis and Represent Therapeutic Targets Jean K. Gustin, Ying Bai and **Ashlee V. Moses**	9.2
09:30	KSHV infection of lymphatic endothelial precursor cells Terri DiMaio and **Michael Lagunoff**	9.3
09:45	Gene Expression Changes That Define the Antiviral Activity of 25-Hydroxycholesterol Against KSHV and EBV Infections **Anna P. Serquiña,** Takanobu A. Tagawa, Daniel Oh, Guruswamy Mahesh, Joseph M. Ziegelbauer	9.4
10:00	BiP Activity Is Critical for KSHV Reactivation and Survival of Latently Infected B Cells **Guillermo Najarro,** Gil Torten[2] Carolina Arias	9.5
10:15	SARS-CoV-2 proteins and anti-COVID-19 drugs induce lytic reactivation of KSHV Jungang Chen, Lu Dai, Lindsey Barrett, Steven Post, **Zhiqiang Qin**	9.6
10:30 – 11:00	**Break**	
11:00 - 12:30	**Therapeutics**	**Session X**
Moderators: **Ethel Cesarman**, Weill Cornell Medical College, United States, and **Laurie Krug,** National Cancer Institute, United States		
11:00	High levels of LINE-1 transposable elements expressed in Kaposi's sarcoma-associated herpesvirus-related primary effusion lymphoma contribute to their proliferation Anuj Ahuja, Guy Journo, Ron Eitan, and **Meir Shamay**	10.1

11:15	Primary effusion lymphoma enhancer connectome links super-enhancers to dependency factors **Chong Wang**, Luyao Zhang, Liangru Ke, Sizun Jiang, Difei Li, Yohei Narita, Isabella Hou, Jun Liang, Shijun Li, Haipeng Xiao, Eva Gottwein, Kenneth M. Kaye, Mingxiang Teng, Bo Zhao	10.2
11:30	XPO1 is a vulnerable target of Kaposi's sarcoma and primary effusion lymphoma, and mediates innate immune response during KSHV primary infection **Wen Meng**, Shou-Jiang Gao	10.3
11:45	Metabolic roles of IKKepsilon in KSHV induced tumor growth **Ali Can SAVAS**, Mehrnaz Zarinfar, Ting-yu Wang, Chao Qin, Youliang Rao, Yongzhen Liu, and Pinghui Feng	10.4
12:00	Inflammasome activation in patients with KSHV-associated disorders **Ramya Ramaswami**, Silvia Lucena Lage, Kathryn Lurain, Joseph Rocco, Maura Manion, Robert Yarchoan, Irini Sereti	10.5
12:15	Targeting Metabolic Vulnerabilities in Primary Effusion Lymphoma Using the Novel Nucleoside Analog (6-ETI) **Jouliana Sadek**, Jan Krumsiek, Tuo Zhang, Barbara Coan, Maite Ibanez de Garao, Tanner Cole, Sara Yahyaei, Herman Van Besien, Ethel Cesarman	10.6
12:30-01:00	**Meeting on Next KSHV Workshop**	

KSHV 2021 Virtual Meeting on
Kaposi's Sarcoma Herpesvirus
(KSHV)
& Related Agents

Session I:

Virus-Host Interactions-I

7:30am-9:00am

1.1

Single-cell analysis of Kaposi's sarcoma herpesvirus infection in three-dimensional air-liquid interface culture model

Kyle L. Jung[1#], Un Yung Choi[1#], Angela Park[2#], Jae U Jung[1]

[1]Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44115. [2]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033.
[#]Authors contributed equally

While the oral cavity has been previously identified as the major site for transmission of Kaposi's sarcoma-associated herpesvirus (KSHV), it remains unclear as to how KSHV is able to establish an infection in the oral epithelia. We have developed a de novo KSHV infection model using fully differentiated, three-dimensional (3D) oral epithelial organoids at an air-liquid interface. Our infection model has revealed that KSHV infects the oral epithelia when the basal epithelial cells are exposed by damaging epithelial barrier. The infection model demonstrates higher levels of spontaneous KSHV reactivation compared to two-dimensional (2D) cell cultures with lytic cells enriched in the superficial layer of the tissue. Single cell RNA sequencing revealed that KSHV infection has a time-dependent effect upon both the infected cells and uninfected bystander cells, affecting processes such as oxidative phosphorylation and epithelial differentiation. Within the infected cells, we also identify a unique viral population with a distinct viral and host gene expression profile compared to latent or lytic KSHV cells. This study demonstrates that our 3D epithelial infection model can be used to gain a better understanding of oral KSHV infection and transmission.

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1.2

Polycomb group proteins prevent aberrant transcription and episome clearance of KSHV

Weissmann Simon[1], Ziegler Marion[1], Guenther Thomas[1], Grundhoff Adam[1]

[1]*Leibniz Institute for Experimental Virology (HPI), Virus Genomics, Hamburg, Germany*

Latency as well as lytic reactivation of KSHV represent processes that are tightly regulated at the epigenetic level, in particular via histone modifications. Tri-methylation of histone H3 at lysine residue 27 (H3K27me3), mediated by the polycomb repressive complex 2 (PRC2), and ubiquitination of H2A lysine 119 (H2AK119Ub), mediated by the polycomb repressive complex 1 (PRC1), are associated with transcriptionally repressed chromatin and can be found on KSHV episomes early after viral entry and chromatinization. Here, we employed genome-editing techniques to establish cellular models of constitutive and inducible PRC1 and PRC2 inactivation. We used these models to investigate the effects of H3K27me3 and H2AK119Ub loss in KSHV biology. We found that deletion of SUZ12, the structural component of the core PRC2 complex, causes a complete loss of H3K27me3. Surprisingly, we observed reduced but still significant levels of H2AK119Ub, arguing that PRC1 can be recruited to KSHV independently of PRC2 activity. Furthermore, we demonstrate that loss of H3K27 methylation leads to histone hyperacetylation of KSHV episomes, supporting an active chromatin state, which ultimately leads to aberrant transcription of broad regions of the viral genome. Interestingly, ablation of PRC activity led to a drastic reduction of latent episome maintenance, resulting in increased episome loss during cell division. Of note, this accelerated loss was independent of changes in cellular proliferation rates or lytic reactivation of KSHV infected cells. Our results therefore suggest that PRCs play a novel and unexpected role during replication and/or segregation of viral episomes. We will discuss the potential mechanisms of viral transcriptional derepression, how the absence of polycomb activity may cause increased KSHV episome clearance, and whether increased viral transcription and episome loss are interdependent.

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1.3

Redefining De Novo Gammaherpesvirus Infection Through High-Dimensional, Single-Cell Analysis of Virus and Host

Jennifer N. Berger[1], Bridget Sanford[2], Abigail K. Kimball[3], Lauren M. Oko[1], Rachael E. Kaspar[3], Brian F. Niemeyer[1], Kenneth L. Jones[4], Linda F. van Dyk[1]*, **Eric T. Clambey**[3]*

*[1]Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus | Aurora, CO, 80045, USA; [2]Department of Pediatric Oncology, University of Colorado Anschutz Medical Campus | Aurora, CO, 80045, USA; [3]Department of Anesthesiology, University of Colorado Anschutz Medical Campus | Aurora, CO, 80045, USA; [4]Department of Cell Biology, University of Oklahoma Health Sciences Center | Oklahoma City, OK, 73104, USA. * Co-corresponding authors.*

Virus infection is frequently characterized using bulk cell populations. How these findings correspond to infection in individual cells remains unclear. Here, we integrate high-dimensional single-cell approaches to quantify viral and host RNA and protein expression signatures using de novo infection with the well-characterized model, murine gammaherpesvirus 68, during lytic replication. While infected cells demonstrated genome-wide transcription, individual cells revealed pronounced variation in gene expression, with only 9 of 80 annotated viral open reading frames uniformly expressed in all cells, and a 1000-fold variation in viral RNA expression between cells. Single-cell analysis further revealed positive and negative gene correlations, many uniquely present in a subset of cells. Beyond variation in viral gene expression, individual cells demonstrated a pronounced, dichotomous signature in host gene expression, revealed by measuring host RNA abundance and post-translational protein modifications. These studies provide a resource for the high-dimensional analysis of gammaherpesvirus infection, and a conceptual framework to define virus infection as the sum of virus and host responses at the single-cell level.

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1.4

Identification of KSHV episome docking sites on host chromosomes and regulation of active KSHV episome maintenance

Ashish Kumar[1], Yuanzhi Lyu[1], Vladimir Majerciak[2], Michelle Salemi[3], Ryan R. Davis[4], Clifford G. Tepper[5,6], Chie Izumiya[1], Mel Campbell[1], Zhi-Ming Zheng[2], & Yoshihiro Izumiya[1,5,6]

[1]Department of Dermatology School of Medicine, University of California Davis (UC Davis), Sacramento, California USA; [2]HIV Dynamic and Replication Program, National Cancer Institute, NIH, Frederick, Maryland, USA; [3]Genome Center, Proteomics Core, Genome and Biomedical Sciences Facility, UC Davis, Davis, California, USA; [4]Department of Pathology and Laboratory Medicine, School of Medicine, UC Davis, Sacramento, California, USA; [5]Department of Biochemistry and Molecular Medicine, School of Medicine, UC Davis, Sacramento, California USA; [6]Viral Oncology and Pathogen-Associated Malignancies Initiative, UC Davis Comprehensive Cancer Center, Sacramento, California USA.

In KSHV- or EBV-infected individuals, the person will carry viral episomal genomes for the remainder of their lives. These latently infected genomes are not permanently silenced, but rather active genomic domains are poised to receive external signals with bivalent histone marks. Such activation through cell signaling stimulates transcription and expands genome-wide for completion of viral lytic replication. The active transcription program is embedded in the genome and thus maintained for very long duration through multiple cell divisions, which is a remarkable epigenetic feature. KSHV episomes are maintained as non-integrated circular genomes and KSHV latency-associated nuclear antigen (LANA) plays an essential role in tethering host chromosomes and silencing viral chromatin.

In this study, we examined the nuclear microenvironment of KSHV episome tethering sites by a combination of Capture Hi-C, proximity biotin-labelling with mini-TurboID-LANA virus, and CUT&RUN. Capture Hi-C identified preferential KSHV episome tethering sites in naturally infected PEL cells and found that episome docking sites are not random, which over 80% of tethering sites were found to be common among three PEL cell lines, BCBL-1, BC1 and BC3. Proximity biotin labeling with mini-TurboID-LANA and subsequent in vitro binding studies identified that LANA interacts with ChAHP complex (CHD4, ADNP and HP1) in latently infected cells, and CUT&RUN confirmed LANA's colocalization with the complex on both KSHV and host cellular chromosomes at epigenetically active docking sites. Knock-down of CHD4 in latently infected cells strongly sensitized KSHV reactivation, while overexpression of CHD4 almost completely diminished K-Rta-mediated KSHV reactivation in iSLK cells. In addition, CHD4 knock-down prevented KSHV entry into latency in 293 cells following de novo infection. Since CHD4 is known to regulate accessibility of cellular enhancers to promoters, our studies suggest that active KSHV latent chromatin is maintained by enhancer-promoter communication, which might be regulated by the ChAHP-LANA interactions at terminal repeats-lncRNA genetic hubs.

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1.5

Exhaustive Functional Tiling of the KSHV Genome Identifies Essential Domains in Proteins Required for Late Gene Transcription

David W Morgens[1], Divya Nandakumar[1], Allison L Didychuk[1], Britt Glaunsinger[1,2,3]

[1]Department of Plant and Microbial Biology, UC Berkeley, Berkeley, CA, USA; [2]Department of Molecular and Cell Biology, UC Berkeley, CA, USA; [3]Howard Hughes Medical Institute, UC Berkeley, CA, USA

While traditional methods for studying large DNA viruses allow the creation of individual mutants, CRISPR/Cas9 can be used to rapidly create thousands of mutant dsDNA viruses in parallel. We designed a sgRNA library containing all ~22,000 guides targeting the ~154 kb Kaposi's sarcoma-associated herpesvirus (KSHV) – one cut site approximately every 8 base pairs – enabling the pooled screening of both coding and non-coding regions. We used this tool to phenotype all possible Cas9-targeted viruses for transcription of KSHV late genes, which is required to produce structural components of the viral capsid. Late gene transcriptional regulation is mechanistically unique and requires at least six KSHV-encoded transcriptional activators to turn on late promoters in a DNA-replication dependent manner. We robustly recovered five of the six components of the viral transcriptional activation complex along with all six known components of the viral DNA replication machinery. We also identified ORF46, which had not been previously linked to KSHV late gene expression. Based on our results along with previous studies of the EBV homolog, we demonstrate ORF46 plays a role in viral DNA replication.

This exhaustive tiling approach gives resolution beyond that of individual viral ORFs, allowing identification of key regions within genes. By performing targeted deep sequencing of the viral genome, we identified and tracked mutations caused by Cas9 in several genes required for late gene expression. This revealed important functional regions of targeted proteins, providing an additional layer of mechanism-orientated information to functional screens. For example, disruptions to the DNA-binding domain but not the catalytic domain of ORF46 prevents DNA replication, consistent with previous data from EBV. This approach, when used with various phenotypic screens as well as alternative variants of Cas9, should allow an exhaustive functional interrogation of the KSHV genome – one that could be extended to other dsDNA viruses.

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1.6

Highly Multiplexed Tissue Imaging of Nucleic Acids and Proteins Resolves the Tissue Immune Organization During Viral Infections

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Orchestrated tissue and immune events are fundamental to our understanding of disease outcomes. A detailed comprehension of complex spatial host-disease interactions *in situ* is essential to develop new anti-viral prevention and therapeutic strategies. Here, we developed Protein And Nucleic acid IN situ Imaging (PANINI) coupled with Multiplexed Ion Beam Imaging (MIBI), a sensitive method for simultaneously quantifying the expression of DNA, RNA, and protein targets to interrogate the cellular composition, variability, and composite microenvironments in tissue compartments. Our initial PANINI-MIBI approach measured >30 parameters simultaneously across large sections of archival lymphoid tissues in Simian Immunodeficiency Virus (SIV) infected and uninfected non-human primates (NHP). This enabled the unprecedented dissection of cellular phenotypes, functional markers, viral DNA integration events, and viral RNA transcripts. Our results reveal purposeful host immune coordination in response to acute and chronic viral infection, uncovering functional roles of the immune environment in association with active and latent infection status. We discovered the unexpected upregulation of IL10 in B cells in response to SIV infection, thereby inducing an immunosuppressive environment conducive to macrophage M2 polarization and viral production. Finally, we describe the microenvironment features that stratify infected and uninfected animals and between latent or active infected cells. The method and data presented herein provide a multi-modal experimental and spatial analytical framework to reveal insights into higher-level spatial coordination during viral infections and are readily applicable to infectious diseases and tumor virus biology.

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KSHV 2021 Virtual Meeting on
Kaposi's Sarcoma Herpesvirus
(KSHV)
& Related Agents

Session II:

Clinical Epidemiology

9:30am-11:00am

2.1

Cytokines, Chemokines, and HIV Viral Load are Associated with Recurrence of HIV-associated Kaposi's Sarcoma

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Kaposi's sarcoma (KS) is an AIDS-defining angio-proliferative malignancy which remains highly prevalent in sub-Saharan Africa (SSA) in spite of anti-retroviral therapy (ART), and chemotherapy is the only available treatment for advanced KS in SSA. Unfortunately, KS recurrence is common in chemotherapy treated patients, and very little is known about the contributing factors or potential biomarkers for disease recurrence. The main objective of this study was to determine the factors associated with recurrence of HIV-associated KS. We recruited a cohort of 39 individuals on antiretroviral therapy who were in remission for HIV-associated KS after undergoing cytotoxic cancer chemotherapy. Twenty-seven (27) of these had sustained KS remission while 12 had KS recurrence during the 12-month follow-up period. Collected variables included sociodemographic and clinical parameters, cytokines and chemokines, HIV viral loads, and CD4 counts. For individuals with sustained remission, IL-5 was significantly higher at time of follow-up (22.7pg/ml *vs.* 2.4pg/ml; p=0.02). Their IL-6 levels were also significantly higher at baseline and time of follow-up, (18.4pg/ml *vs.* 0pg/ml; p=0.01) and (18.0pg/ml *vs.* 0.18pg/ml; p=0.03) respectively. Whereas, their IP-10 was significantly lower at baseline and at time of follow-up in individuals (920pg/ml *vs.* 534pg/ml; p=0.04) and (1098pg/ml *vs.* 446pg/ml; p=0.01) respectively, as compared to those with recurrent KS. Their HIV viral load was also found to be significantly lower at baseline and at time of follow-up, (113copies/ml *vs.* 0copies/ml; p=0.004) and (152copies/ml *vs.* 0copies/ml; p=0.025), respectively. In conclusion, plasma levels of IL-5, IL-6, and IP-10 could be biomarkers associated with recurrence of HIV-associated KS, and persistently detectable HIV viral loads increase the risk of KS recurrence.

Key Words: HIV, Kaposi's Sarcoma, Antiretroviral Therapy, Cancer Chemotherapy, Recurrence

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2.2

The contribution of KSHV and/or EBV co-infection to COVID-19 severity and outcome in South African patients

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In South Africa, the COVID-19 pandemic is occurring against the backdrop of high HIV, tuberculosis and non-communicable disease burdens as well as prevalent herpesviruses infections such as Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). As part of an observational study of adults admitted to Groote Schuur Hospital, Cape Town, South Africa during the period June – August 2020 and assessed for SARS-CoV-2 infection, we measured KSHV serology and KSHV and EBV viral load in peripheral blood in relation to COVID-19 severity and outcome. In total, 154 patients were recruited, 54% men, 46% women with a median age of 52 years (range: 20 – 86). 30.5% (95% CI: 23.4 – 38.4%) of the cohort was HIV positive and 40.4% (95% CI: 32.3 – 49.0%) were KSHV seropositive. EBV viral load was detectable in 82% (95% CI: 74.3 – 88.3%) of the cohort while KSHV DNA was detected in 31.5% (95% CI: 24.2 – 39.7%), with dual infection in 21.1% (95% CI: 14.4 – 29.2%). Overall, 104 patients (67.5% (95% CI: 59.5 – 74.8%)) had a PCR-confirmed SARS-CoV-2 infection, 30 (28.8% (95% CI: 20.4 – 38.6%) of whom died. In COVID-19 patients, detectable KSHV viral load was associated with death (WHO ordinal scale level 8) after adjusting for age, sex, HIV status and detectable EBV VL (p=0.036, adjusted OR=3.17 [95% CI: 1.08 – 9.32]). While the design of our study cannot distinguish if disease synergy exists between COVID-19 and KSHV nor if either viral infection is indeed fueling the other, these data point to a potential contribution of KSHV infection to COVID-19 outcome, particularly in the South African context of high disease burden, that warrants further investigation.

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2.3

International epidemiology of KSHV in HIV-1 infected individuals on cART

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KSHV/HIV co-infected persons are at high risk for KS, the incidence of which declined dramatically upon cART introduction, but did not decrease subsequently. It is unclear whether the epidemiology of KSHV changed after cART introduction. We investigated the issue in the PEARL (ACTG A5175) clinical trial (NCT00084136), which recruited patients from diverse countries, mainly in resource limited settings. For each participant, we tested the first and last sera available between 2006 and 2010 for IgG to KSHV, using ELISAs based on recombinant K8.1 and LANA. The analysis included 1276 individuals (46% women) from the USA (412), Brazil (448), Haiti (200), Peru (266), Malawi (434), South Africa (378) Zimbabwe (76), Thailand (200). At baseline, the mean age was 36 years (SD 9), the mean CD4+ count was 160 cells/mL (SD 85) and the mean HIV load was 5 log RNA copies/mL (SD 0.66). KSHV seroprevalence varied between 25% and 76%. The following risk factors were considered: age (below or above 30), sex, study site country, baseline CD4+ (below or above 150), baseline HIV VL (below or above 5 logs). When controlling for all other covariates, male sex (OR 1.59, 95%CI 1.33-1.9) African site (OR 2.8 95%CI 2.33-3.35) and baseline CD4+ (1.21, 95%CI 1.02-1.44) were significantly associated with prevalent KSHV infection. Mean follow-up was 3.52 years (SD 1.07). KSHV seroincidence was 3.6/100 p/y (4.4 in men, 2.8 in women). Multivariate analysis included the above-mentioned covariates and CD4+ count change between entry and exit testing (≤0 or >0). The following possible protective or risk factors for incident KSHV infection emerged: male sex (IRR 1.67, 95%CI 1.17-2.35) and age over 30 (IRR 0.67, 95%CI 0.48-0.93). In summary, KSHV prevalence was very variable geographically, but the incidence was high overall, particularly in younger men.

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2.4

Serial Profiling of Tumor-Infiltrating Lymphocytes in Kaposi's Sarcoma

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OBJECTIVE: Kaposi sarcoma (KS) tumors contain abundant T- and B-lymphocytes but their influence on the natural history of KS is undefined. We are performing single-cell RNA sequencing (scRNAseq) to define the transcriptional profile of tumor-infiltrating T- and B-cells in KS tumors to determine if the profile changes over time and whether it is associated with clinical outcome.
METHODS: Serial blood samples, tumor biopsies (up to 3 biopsies from different skin sites at each visit), and a biopsy of normal skin are obtained from HIV^+ and HIV^- adults presenting to the Uganda Cancer Institute with KS. ScRNAseq is performed on lymphocytes from blood and from single-cell suspensions prepared from tumors. The uniquely rearranged antigen receptor sequences carried in T- and B-cells enable serial monitoring of the transcriptional profile of specific T- and B-cell clones over space (*i.e.*, tumors from different positions on the body) and time.
RESULTS: T- and B-lymphocytes infiltrating KS tumors are highly oligoclonal, suggesting selective migration into or proliferation within the tumor microenvironment. Specific T- and B-cell clones identified by their unique antigen receptor sequences can be identified in multiple synchronously acquired but non-contiguous tumors, as well as in tumors acquired at different times. Dominant T- and B-cell clones in KS tumors are also often detected in the blood at high frequency. These observations support the hypothesis that KS tumor-infiltrating T- and B-cells comprise a systemic tumor-reactive adaptive immune response. A range of transcriptional phenotypes are identified in KS tumor-infiltrating lymphocytes, and the phenotypes carried in individual T- and B-cell clones varies across both space and time.
CONCLUSIONS: ScRNAseq of T- and B-lymphocytes from KS tumors and blood supports the existence of a systemic tumor-reactive immune response. We are currently exploring whether this response is associated with clinical outcome, and whether it might be manipulated for therapeutic benefit.

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2.5

Whole-genome sequencing of KSHV from patient samples: Evidence for a distinctly African Lineage

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KSHV was first sequenced from a PEL derived cell line, BC-1 and subsequently, the KSHV genomic architecture has been well characterized. Here we report on the genome sequencing of two KSHV genomes isolated from PEL patient samples obtained from the AIDS and Cancer Specimen Repository, and 10 archival KS and Saliva samples. To also cover the virus, repeat regions, which are typically simply copied from the reference, we designed primers to amplify these functionally important GC rich regions. These regions include the terminal repeats, the LANA repeats, and the origins of replication. We also sequenced and compare multiple BAC isolates of KSHV to estimate background mutations rates in the absence of human transmission. The evolutionary relationship of the polymorphic K1 gene of our isolates and 137 publicly available KSHV genomes, reveal the distinct geographical clustering of KSHV, except for several outliers. In contrast, the evolutionary relationship of the KSHV based on the region between the 2 origins of replication in the long unique coding region, reveals the distinct clustering of the African isolates from the European and western isolates. Notably, the sequences for the African isolates were evolutionary distant from the BAC constructs commonly used to study KSHV *in vitro*.

The inclusion of a larger number of KSHV genomes in our phylogenetic analysis, compared to previous studies has demonstrated a substantial change in the evolutionary relationships of KSHV. These findings strongly supported the migration patterns and founder effects observed out of Africa and necessitate for increased sequencing of KSHV isolated from Africa, to gain a deeper understanding of KSHV diversity, evolution, and functioning. Furthermore, our findings indicate that a more genetically relevant BAC construct needs to be engineered to be more inclusive of the genetic variability observed in KSHV isolated from Africa.

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2.6

Immune and Virus-specific Responses in Participants with Primary Effusion Lymphoma Receiving Lenalidomide, Dose-adjusted EPOCH, and Rituximab (EPOCH-R^2)

Kathryn Lurain[1], Romin Roshan[2], Ramya Ramaswami[1], Ralph Mangusan[1], Anaida Widell[1], Irene Ekwede[1], Joseph Ziegelbauer[1], Thomas S. Uldrick[1], Denise Whitby[2], Robert Yarchoan[1], and Laurie T. Krug[1]

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In a prospective trial, 6 HIV$^+$ participants with PEL received antiretroviral therapy and 6 cycles of lenalidomide, etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone, and rituximab. 3 had complete responses (responders); 3 had progressive/stable disease (non-responders). We performed flow cytometry evaluating T-cell subsets, monocytes, and neutrophils at baseline, post-cycle 1 (C1), post-cycle 4 (C4), end-of-treatment, and 1-year post-end-of-treatment (YR1). KSHV and CMV/EBV/influenza-specific T-cell responses were measured using an interferon-gamma ELISPOT assay at baseline, end-of-treatment, and YR1. We determined breadth of virus-specific responses by number of KSHV ORFs eliciting interferon-gamma responses and intensity by mean spot forming units per 10^6 PBMCs. Comparisons were evaluated by Wilcoxon rank sum and Spearman correlation. At baseline, responders had a higher percentage of inflammatory tissue factor (TF)$^+$ CD14$^+$ monocytes compared with non-responders (median 30% vs 10%, p=0.049), higher percentage of PD-1$^+$ CD4$^+$ effector memory (CD27$^-$CD45RO$^+$) T (TEM)-cells (median 90% vs 71%, p=0.049), and lower percentage of CD38$^+$ CD8$^+$ TEM-cells (median 75% vs 96%; p=0.049). Responders had a decrease in TF$^+$ CD14$^+$ monocytes from baseline to C1 and C4 (p=0.049, 0.049) and decrease in PD-L1$^+$ CD14$^+$ monocytes from baseline to C1 (p=0.049) compared to non-responders. PD-L1 expression on monocytes and neutrophils was strongly correlated (r=0.74), and TF and PD-L1 expression on monocytes was correlated (r=0.45) when analyzing end-of-treatment and YR1 changes from baseline. KSHV ORF reactivity was variable with no differences in breadth or intensity between response groups. KSHV-specific responses strongly correlated with end-of-treatment and YR1 changes from baseline in CD8$^+$ counts (r=0.86) and negatively correlated with end-of-treatment and YR1 changes from baseline in PD-L1 expression on monocytes and neutrophils (r=-0.57, -0.69). Changes in TF and PD-L1 expression on monocytes and PD-1 expression on CD4$^+$ TEM-cells may reflect immune activation and inflammation and predict outcomes in PEL. More data are needed to understand how virus-specific responses affect outcomes.

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Session III:

Immunity and Vaccine

11:30am-1:00pm

3.1

ADAR1 Facilitates KSHV Lytic Reactivation by Modulating the RLR-Dependent Signaling pathway

Huirong Zhang[1], Guoxin Ni[1], and Blossom Damania[1]

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KSHV replication leads to production of virus- and host-derived RNAs. Some of the RNAs contain RNA duplexes that can be recognized by host RNA sensors such as retinoic acid inducible gene I (RIG-I) and melanoma differentiation associated gene 5 (MDA5). Upon activation, RIG-I and MDA5 interacts with the adaptor protein mitochondrial antiviral signaling (MAVS), inducing RIG-I-like receptor (RLR) signaling pathway and subsequent type I IFN production. The process establishes an antiviral state and inhibit KSHV infection and replication. Adenosine deaminase acting on RNA 1 (ADAR1), a dsRNA adenosine (A)-to-inosine (I) editing enzyme, plays a critical role in avoiding autoinflammation by preventing RNA sensors from recognizing host-derived RNAs that contain RNA duplexes. In our study, we found ADAR1 is required for optimal KSHV lytic replication from latency. Knockdown of ADAR1 in KSHV latently infected cells inhibits viral gene transcription and viral replication during KSHV lytic reactivation. ADAR1 deficiency also significantly increases type I interferon production during KSHV reactivation. This increased interferon response is dependent on activation of the RLR signaling pathway. Depletion of ADAR1 together with either RIG-I, MDA5, or MAVS reverses the increased IFNβ production and rescues KSHV lytic replication. These data suggest that ADAR1 serves as a proviral factor for KSHV lytic reactivation and facilitates DNA virus reactivation by dampening the RLR pathway-mediated innate immune response.

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3.2

N6-methyladenosine (m6A) mediates Innate Immune Response During Bacterial and Viral Infections

Jian Feng[1], Teng Zhang[2], Océane Sorel[3], Wen Meng[1], Xinquan Zhang[1], Yidong Chen[4], Yufei Huang[2,4] and Shou-jiang Gao[1,3]

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N^6-methyladenosine (m^{6}A) is a dynamic post-transcriptional RNA modification influencing all aspects of mRNA biology, including biogenesis, splicing, export, translation and decay. m^{6}A modifications have been profiled during infections of numerous viruses. However, the role of m^{6}A in innate immune response has not been systematically described. Here, we examined the cellular m6A epitranscriptomes during infections of bacteria, herpes simplex virus (HSV) and KSHV as well as treatment with lipopolysaccharide (LPS) in an effort to identify common and distinct innate immune response genes mediated by m^{6}A. We showed that a significant portion of cellular genes underwent 5'UTR hypomethylation and 3'UTR hypermethylation during viral and bacterial infections, and LPS treatment, many of which were innate immune response genes. We identified common and distinct m^{6}A-modified genes under different stimulating conditions. Several sets of genes whose expression levels were correlated with m^{6}A peak changes, suggesting regulation of these genes by m^{6}A modifications. To confirm the roles of m^{6}A modifications in gene expression, we performed knockdown of ALKBH5, a "eraser" of m^{6}A during *Pseudomonas aeruginosa* infection and identified genes that had significant methylation enrichments. Among the innate immune response genes that were regulated by m^{6}A modifications, we confirmed a set of genes including TNFAIP3, IFIT1, IFIT2, and IFIH1 by independent examination of m^{6}A modifications and gene expression changes. Taken together, our results revealed that m6A modifications play vital roles during innate immune responses against bacterial and viral infections. These works also provided rich resources for the scientific community.

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3.3

Viral miRNA target identification reveals a surprising role for EWSR1 in germinal center B cell responses

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Germinal center (GC) B cells represent both a critical stage of gammaherpesvirus-driven B cell differentiation and an important point of vulnerability for the genesis of numerous types of B cell lymphomas. Gammaherpesviruses employ numerous molecular mechanisms, including encoding miRNAs, to manipulate infected B cells and drive them through GC reactions. Although numerous host mRNA targets of gammaherpesvirus miRNAs have been identified, the specific *in vivo* functions of targeted mRNA repression remain unknown due to strict species restriction. Infection of mice with murine gammaherpesvirus 68 (MHV68) provides a robust virus-host system to dissect the *in vivo* function of conserved gammaherpesvirus genetic elements. Here, we determined that an MHV68 mutant deficient in *mghv-miR-M1-7* (*miR-7*) was significantly attenuated for infection of GC B cells. Following the use of qCLASH (quick cross-linking, ligation, and sequencing of hybrids) to identify targets of *miR-7*, we used highly specific shRNA-mediated *in vivo* repression of a single host mRNA target, EWSR1 (Ewing sarcoma breakpoint region 1), to demonstrate that *miR-7* targeting of EWSR1 is critical for GC B cell infection. Consistent with this, we found that B cell specific EWSR1 deletion resulted in a nearly complete restoration of GC B cell infection. Intriguingly, ablation of EWSR1 in B cells resulted in a striking increase in the number of GC B cells and in the production of antigen-specific IgG in response to T cell-dependent non-virus antigen stimulation. These findings reveal EWSR1 as a novel host mediator of gammaherpesvirus-driven and antigen-mediated GC B cell responses.

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3.4

Vaccination with a Replication-Dead Gammaherpesvirus Protects against Wild-Type Virus Replication, Reactivation, and Disease in Mice

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The gammaherpesviruses are oncogenic viruses that establish lifelong infections. While several vaccine strategies to limit gammaherpesvirus infection and disease are in development, there are no FDA-approved vaccines for Epstein-Barr virus or Kaposi sarcoma herpesvirus. Using murine gammaherpesvirus-68 (MHV68), a mouse model for gammaherpesvirus pathogenesis studies, we previously developed a codon-shuffling-based complementation strategy that enabled the production of replication-dead MHV68 (RDV). This method prevents recombination with the helper gene and reversion to virulence in the RDV vaccine due to WT virus contamination. We generated an RDV lacking expression of the essential replication and transactivator protein (RTA) to arrest viral gene expression early after de novo infection. Inoculation with RDV-RTA exposes the host to intact virion particles and leads to limited lytic gene expression in infected cells. Prime-boost vaccination of WT C57BL/6 mice with RDV-RTA elicited virus-specific neutralizing antibody and effector T cell responses in the lung and spleen tissues. In vaccinated mice challenged intranasally with WT MHV68, there was near complete abolishment of virus replication in the lungs 7 days post-challenge and virus reactivation from spleens 16 days post-challenge. IFNAR$^{-/-}$ mice, which lack the type I interferon receptor, exhibit severe disease upon infection with WT MHV68. RDV-RTA vaccination of IFNAR$^{-/-}$ mice prevented wasting and mortality upon challenge with 2x10^6 PFU of WT MHV68. These results demonstrate that prime-boost vaccination with a gammaherpesvirus that is disabled for lytic replication offers protection against acute replication, reactivation, and severe disease upon WT challenge.

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3.5

Investigating the activation of host caspases during KSHV lytic infection as a mechanism of immune evasion

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KSHV has evolved an arsenal of immune evasion strategies, including many that target the type I interferon (IFN) response, to successfully replicate in a host. While there are many known anti-IFN factors encoded by KSHV, our lab has revealed that KSHV also exploits the cellular proteases caspases, particularly caspase-8, to block type I IFN responses after lytic reactivation and promote viral replication. This pro-viral role was surprising since caspase-8 activation typically elicits cell death, which is generally considered anti-viral. We are interested in understanding how lytic reactivation triggers caspase-8 activation and hijacks its activity to support viral replication. We surprisingly found that in this context, caspase-8 activation may not require canonical death receptor signaling, since caspase-8 is still activated upon knock-down of FADD, a key adaptor protein in the death receptor pathway. In contrast, TRIF, an adaptor protein that associates with the Toll-like receptors TLR3 and TLR4, is involved in caspase-8 activation during KSHV lytic reactivation, as TRIF knock-down reduces levels of cleaved caspase-8. Preliminary data also suggest a role for TLR3 in caspase-8 activation during KSHV lytic reactivation. These results suggest that viral sensing through the TLR3-TRIF pathway may drive caspase-8 activation during KSHV lytic infection. Additionally, our lab has found that caspase activity suppresses IFN induction by targeting a different viral sensing pathway, the DNA sensing cGAS pathway. When stimulated, the DNA sensor cGAS catalyzes 2'3'-cGAMP to mediate IFN signaling. Our data suggest that caspase activity suppresses type I IFN signaling during KSHV lytic reactivation by inhibiting cGAS activity and 2'3'-cGAMP production. Thus, KSHV may exploit activation of the TLR3 pathogen sensing pathway to inhibit anti-viral DNA sensing. Further elucidation of caspase-8 activation during KSHV lytic reactivation will advance our understanding of KSHV-mediated immune evasion and of crosstalk between different innate immune pathways.

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3.6

Kaposi's sarcoma-associated herpesvirus (KSHV) specific IFN-γ T cell responses in HIV seronegative individuals from rural Uganda

Angela Nalwoga[1,2], Romin Roshan[3], Kyle Moore[3], Vickie Marshall[3], Wendell Miley[3], Nazzarena Labo[3], Marjorie Nakibuule[1], Stephen Cose[1,4], Robert Newton[5], Rosemary Rochford[2] and Denise Whitby[3]

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T cell responses to Kaposi sarcoma-associated herpesvirus (KSHV) play a major role in protection from KSHV-associated disease but they have not been well characterised. KSHV prevalence in the rural Uganda is very high (>90%). We investigated IFN-γ T cell responses to the entire KSHV proteome in HIV-negative individuals from a rural Ugandan population cohort. An *ex-vivo* IFN-γ ELISpot assay was carried out on peripheral blood mononuclear cells (PBMC) from 116 individuals, using 85 KSHV overlapping peptide pools tiling 83 KSHV ORFs. KSHV viral load in PBMC was determined using real-time PCR. The intensity of KSHV specific T cell IFN-γ responses were very low, heterogeneous and with little evidence of immune dominance; 38% of the individuals tested reacted to the KSHV K8.1 antigen and 21% reacted to ORF73 antigen. These are the most widely used KSHV antigens. In contrast, IFN-γ responses to Epstein-Barr virus (EBV), Cytomegalovirus (CMV) and influenza peptides were frequent and strong. Our data are unique and suggest that KSHV, amongst the 8 human herpesviruses, has unique drivers for control that we are still yet to understand. Our study is the most comprehensive yet conducted and the results provide a basis for further investigation of KSHV T cell responses in immune-competent individuals.

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Session IV:

Virus-Host Interactions-II

7:00am-8:30am

4.1

NDRG1 facilitates lytic replication of Kaposi's sarcoma-associated herpesvirus by maintaining the stability of the KSHV helicase

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The presumed DNA helicase encoded by ORF44 of Kaposi's sarcoma-associated herpesvirus (KSHV) plays a crucial role in unwinding viral double-stranded DNA and initiating DNA replication during lytic reactivation. However, the regulatory mechanism of KSHV ORF44 has not been fully elucidated. In a previous study, we identified that N-Myc downstream regulated gene 1 (NDRG1), a host scaffold protein, facilitates viral genome replication by interacting with proliferating cell nuclear antigen (PCNA) and the latent viral protein latency-associated nuclear antigen (LANA) during viral latency. In the present study, we further demonstrated that NDRG1 can interact with KSHV ORF44 during viral lytic replication. We also found that the mRNA and protein levels of NDRG1 were significantly increased by KSHV ORF50-encoded replication and transcription activator (RTA). Remarkably, knockdown of NDRG1 greatly decreased the protein level of ORF44 and impaired viral lytic replication. Interestingly, NDRG1 enhanced the stability of ORF44 and inhibited its ubiquitin-proteasome-mediated degradation by reducing the polyubiquitination of the lysine residues at positions 79 and 368 in ORF44. In summary, NDRG1 is a novel binding partner of ORF44 and facilitates viral lytic replication by maintaining the stability of ORF44. This study provides new insight into the mechanisms underlying KSHV lytic replication.

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4.2

Kaposi's sarcoma-associated herpesvirus processivity factor (PF-8) promotes PARP1 degradation and lytic replication by recruiting cellular E3 ubiquitin ligase CHFR

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Nuclear enzyme poly (ADP-ribose) polymerase 1 (PARP1) is a multifunctional host factor that catalyzes the polymerization of ADP-ribose units on target proteins. In Kaposi's sarcoma–associated herpesvirus (KSHV)-infected cells, PARP1 inhibits replication and transcription activator (RTA), a molecular switch that initiates lytic replication, through direct interaction. Thus, for efficient replication, KSHV must overcome the molecular barrier in the form of PARP1. Previously, we have demonstrated that KSHV downregulates the expression of PARP1 through PF-8, a viral processivity factor. PF-8 induces ubiquitin–proteasome system–mediated degradation of PARP1 via direct physical association and enhances RTA transactivation activity. Here, we showed that dimerization domains of PF-8 are crucial not only for PARP1 interaction and degradation but also for enhancement of the RTA transactivation activity. PF-8 alone did not induce DNA damage response (DDR) and physical association of PF-8 and PARP1 was not affected by an ATM inhibitor, suggesting that PF-8-induced PARP1 degradation is unlikely due to an indirect effect of DDR. PF-8 recruited checkpoint with FHA and RING finger domains (CHFR) for the PARP1 degradation. A knockdown of CHFR attenuated the PF-8–induced PARP1 degradation and enhancement of the RTA transactivation activity, leading to reduced KSHV lytic replication. These findings reveal a mechanism by which KSHV PF-8 recruits a cellular E3 ligase to curtail the inhibitory effect of PARP1 on KSHV lytic replication.

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4.3

A Cytoplasmic Histone Deacetylase Regulates Reactivation of Kaposi's Sarcoma-associated Herpesvirus

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Kaposi's Sarcoma-associated herpesvirus (KSHV) is the etiologic agent of Kaposi's Sarcoma (KS) and other AIDS-related cancers. Reactivation of KSHV from latency requires expression of the viral protein Rta and is a necessary step for progression of these diseases. Host cell histones play a major role in maintaining latency of KSHV as a nuclear DNA episome. We previously showed positive and negative effects of various histone deacetylases (HDACs) on virus infection: inhibition of HDAC classes I and IIa with valproic acid (VPA) and maintenance of HDAC6 (class IIb) were optimal for viral reactivation. HDAC6 is a key regulator of protein degradation and cytoskeletal dynamics that functions primarily in the cytoplasm of eukaryotic cells. We found that VPA-induced reactivation is not affected when the HDAC6-specific inhibitor Tubacin is added simultaneously. However, inhibition of HDAC6 as early as 1h post-VPA greatly increases Rta expression and production of infectious virus. Thus, we hypothesize that HDAC6 represses Rta expression in a post-transcriptional manner. Our imaging and cell fractionation experiments show that the majority of HDAC6 is found in the cell cytoplasm, and the small amount of nuclear HDAC6 is reduced by VPA alone or combined with Tubacin. On Western blots, we also observe a low molecular weight protein that is recognized by the HDAC6 antiserum and is only present in infected cells. The major cellular target of HDAC6's deacetylase activity is the alpha-tubulin subunit of microtubules, and we observe that overexpression of an acetyl-alpha-tubulin mimetic protein significantly enhances VPA-induced viral reactivation in Vero cells, recapitulating Tubacin's effect. Overall, our data indicate that HDAC6's effect on microtubule acetylation regulates successful KSHV reactivation from latency. We are currently using CRISPR/Cas9 to delete HDAC6 in infected cells, so we can eliminate its interference with functional studies of ectopic HDAC6.

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4.4

An in-depth analysis of the subcellular localization of the KicGAS (KSHV inhibitor of cGAS) protein in KSHV

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Kaposi's sarcoma-associated herpesvirus (KSHV) is a gamma-herpesvirus that causes Kaposi's sarcoma (KS) and lymphoproliferative diseases. We reported KicGAS (KSHV inhibitor of cGAS) to inhibit cGAS (cyclic GMP-AMP synthase), the principal sensor of cytosolic DNA. Compared to its target protein cGAS, whose intracellular localization is closely regulated, KicGAS is rather small (14 kDa) but remains excluded from the nucleus. As a late-phased lytic protein in KSHV, we speculate its controlled localization to be important for the proper functioning of KicGAS. We first performed extensive microscopy and located a region near the N-terminus that is critical for the exclusively cytoplasmic localization of KicGAS. Further analyses revealed two CRM1-dependent nuclear export signals (NESs), one at the designated region satisfying a PIK NES and functionally more important. Similar NESs were also identified in other γ-2 (RRV, MHV68, HVS, EHV-2, and BHV-6), but not in γ-1 herpesviruses (EBV, MaHV4). Point mutation at the nuclear-localized EBV ORF52 (BLRF2) at S36 to hydrophobic residues enables relocations to the cytoplasm, suggesting the designated area to be essential for the localization of gammaherpesviral ORF52s, but evolutionarily bifurcated at subgenus level. Interestingly, we also observed inconsistencies in response to the CRM1-inhibitor Leptomycin B (LMB) between KicGAS and its truncational mutants, as well as among its homologues, suggesting additional controls to the localization that are specific to KSHV. Finally, we screened for KicGAS mutants in inhibition of cGAS-mediated-IFN response and observed the key residues for NES to be required. Besides, we utilized nuclear export reporter assays and noticed potential interaction between KicGAS and the nuclear exporter CRM1, suggesting multifunctional roles of KicGAS in the KSHV life cycle.

Keywords: KSHV, cGAS, KicGAS, Nuclear export signal, CRM1, oncogenesis

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4.5

Interferon-induced transmembrane proteins inhibit infection by the gamma2-herpesviruses KSHV and RRV in a cell type-specific manner

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The interferon-induced transmembrane proteins (IFITMs) IFITM1, IFITM2, and IFITM3 exhibit antiviral activity towards a broad variety of enveloped viruses at the step of cellular entry. We analyzed the contribution of these proteins to infection by the Kaposi's sarcoma-associated herpesvirus (KSHV) and the related rhesus monkey rhadinovirus (RRV). We used simultaneous CRISPR/Cas9-mediated knockout of IFITM1/2/3 to assess their combined effects on infection, virus trafficking, and membrane fusion, as well as overexpression of individual IFITMs to assess their effects individually. We found that infection by both KSHV and RRV is sensitive to interferon pre-treatment of target cells, which induces IFITMs as does KSHV infection. Knockout of the three IFITMs almost completely reversed the inhibitory effect of interferon in A549 cells, implicating IFITMs as a major defense mechanism in A549. In human fibroblasts, IFITM knockout reversed the inhibitory effect of interferon treatment to a much lesser degree. Preliminary data with human umbilical cord endothelial cells (HUVEC) indicate that, while sensitive to interferon, infection of HUVEC is not affected by IFITMs, suggesting that differences in the viral entry pathway between cells may govern susceptibility to IFITM-mediated restriction. Mechanistically, we found that individual overexpression of IFITM1 but not IFITM2/3 inhibits infection by KSHV and RRV in a cell type-dependent manner. IFITM1/2/3 knockout did not significantly alter expression of the known KSHV cell surface receptors integrin alphaV and EphA2. We did not observe pronounced accumulation of viral particles in the vicinity of IFITMs as was reported for influenza virus. Analyzing cell-cell fusion, we observed that knockout of IFITM1/2/3 in target cells increased fusion activity with effector cells bearing KSHV and RRV glycoproteins. Taken together, our data suggest that KSHV and RRV are inhibited by IFITMs at the step of membrane fusion in a cell type-dependent manner, with IFITM1 being the main effector.

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4.6

Establishing the role of plasma cells in early KSHV infection in B lymphocytes

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Despite decades of research, the mechanisms underlying person-to-person transmission of KSHV remain obscure. Moreover, we have limited information about how the virus establishes infection in a new human host and how it reaches disease-relevant cell types such as B cells and endothelial cells. Given that saliva is widely accepted as the vehicle for KSHV transmission, the oral mucosa represents a likely site for initial infection events and in this context the tonsil and other oral lymphoid tissues represent a likely site for the initial invasion of the immune system by KSHV. In our laboratory, we use a novel ex vivo culture model of human tonsil-derived lymphocytes to mechanistically characterize the establishment of KSHV infection in B cells. We have recently discovered that CD138+ plasma cells, despite being relatively rare in tonsil, represent a highly targeted cell type in early KSHV infection. In this study, we begin to characterize the contribution of plasma cell infection to the overall establishment of KSHV infection in B lymphocytes. We will present recent data using KSHV mutant viruses, as well as manipulation of host cells and cytokines which collectively demonstrate that the targeting of plasma cells influences the magnitude and dynamics of early KSHV spread within B cells. Collectively, our results indicate that factors which limit plasma cell targeting in early infection inhibit the overall establishment of KSHV infection in B cells, an observation which has significant implications for KSHV transmission.

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KSHV 2021 Virtual Meeting on
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Session V:

Processes of Virus Latency

9:00am-10:30am

5.1

The Role of Subnuclear Compartments in KSHV latency establishment

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The role of nuclear compartments in the transcriptional suppression of latent herpesviruses is poorly understood. Recently, we established a novel dCas9-based imaging system for live tracking of Kaposi Sarcoma-associated Herpesvirus (KSHV) episomes. We observed that newly infecting episomes are retained at the nuclear periphery until host cell division, and consistently fail to co-localize with PML bodies, TRIM-defined sub-nuclear compartments that are thought to play an important antiviral role. To investigate the role of sub-nuclear compartments in latency establishment, we exploited the powerful system for programmable 3D Genome Organization, *CRISPR-GO*, to exert programmable control over spatial KSHV episome localization. The system is based on abscisic acid (ABA)-inducible dimerization between compartment-specific proteins and dCas9. By targeting the latter to the terminal repeats (TR) of KSHV, we can reposition KSHV genomes to selected sub-nuclear compartments in an inducible and reversible manner. The efficiency of the system was validated via high-resolution imaging. Complementary analysis of gene expression and chromatin states were also performed. We demonstrate stable and mitosis-independent colocalization of dCas9 and PML or LaminB as early as 3h post-induction, whereas for other proteins (Cenp-A) stable colocalization required cell division and was not observed until approximately 24h post-induction. Interestingly, forced repositioning of KSHV genome to PML, a known modulator of viral chromatin and gene expression of alpha- and betaherpesviruses, efficiently prevented LANA expression and latent infection. ChIP experiments suggest that altered chromatinization of viral episomes is responsible for this phenotype. The CRISPR-GO method facilitates a deeper understanding of latent KSHV chromatin assembly. Our results show that PML bodies, structures thought to primarily restrict lytic infection, can also efficiently counteract gene expression of latently infecting KSHV episomes. We will discuss potential mechanisms and implications for our understanding of PML recognition and escape in viral infection control.

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5.2

KSHV-encoded vCyclin can modulate HIF1α levels to promote DNA replication in hypoxia

Rajnish Kumar Singh[1,5], Yonggang Pei[1], Dipayan Bose[1], Zachary L Lamplugh[1], Kunfeng Sun[1], Yan Yuan[2], Paul Lieberman[3], Jianxin You[4], and Erle S Robertson[1*]

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The cellular adaptive response to hypoxia, mediated by high HIF1α levels includes metabolic reprogramming, restricted DNA replication and cell division. In contrast to healthy cells, the genome of cancer cells, and Kaposi's sarcoma associated herpesvirus (KSHV) infected cells maintains replication in hypoxia. We show that KSHV infection, despite promoting expression of HIF1α in normoxia, can also restrict transcription activity, and promoted its degradation in hypoxia. KSHV-encoded vCyclin, expressed in hypoxia, mediated HIF1 cytosolic translocation, and its degradation through a non-canonical lysosomal pathway. Attenuation of HIF1α levels by vCyclin allowed cells to bypass the block to DNA replication and cell proliferation in hypoxia. These results demonstrated that KSHV utilizes a unique strategy to balance HIF1α levels to overcome replication arrest and induction of the oncogenic phenotype, which are dependent on the levels of oxygen in the microenvironment.

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5.3

A KSHV LANA repeat element demonstrates selective binding and mediates viral persistence

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Kaposi's sarcoma-associated herpesvirus (KSHV) LANA (kLANA) and murine gammaherpesvirus 68 (MHV68) LANA (mLANA) mediate episome persistence for each respective virus during latency. To persist during latent infection, viral, episomal, genomes replicate and then segregate to progeny cell nuclei. Carboxy-terminal LANA comprises the DNA binding domain (DBD) for kLANA and mLANA. The DBD recognizes specific sequence in KSHV or MHV68 terminal repeat (TR) DNA. Carboxy-terminal LANA is essential for LANA mediated DNA replication and episome persistence. Amino-terminal kLANA binds mitotic chromosomes by recognizing histones H2A/H2B on the nucleosome surface. LANA simultaneously binds mitotic chromosomes and viral TR DNA, thereby serving as a molecular tether to segregate episomal DNA to daughter cell nuclei. kLANA and mLANA contain regions of homology, and have a high level of DBD structural homology, but, also have significant differences including in size and sequence. Despite these differences, kLANA and mLANA are capable of acting on each other's TR DNA to mediate episome persistence, and recombinant MHV68 with kLANA substituting for mLANA can establish latency infection in vivo in mice. We have now identified an internal kLANA repeat element which specifically recognizes unacetylated ligand. This element is critical for viral persistence, and for the establishment of latency of chimeric virus containing kLANA in vivo.

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5.4
Identification of Nitro tyrosine and LANA Nuclear Bodies in KS Tumors

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KSHV pathogenesis correlates with KSHV episome persistence. However, most studies on KSHV persistence are conducted in cell culture models and it is not clear how well these studies correspond to KSHV episome persistence in KSHV-infected tumor tissue. In cell culture models, KSHV episome maintenance involves the formation of LANA-KSHV associated higher-order structures, including LANA nuclear bodies (LANA-NBs). We have previously shown that LANA-NBs depend, in part, on the LANA DNA binding domain (DBD) oligomerization and on liquid-liquid phase separation dynamics. To better understand the behavior of LANA and KSHV episomes in KS tumors, we performed RNA-Seq from KS tumor biopsies to identify genes differentially regulated in tumors relative to non-tumor control skin. We now examine LANA nuclear bodies (LANA-NBs) in KS tumor tissue sections and assay for colocalization with factors found to be upregulated in KS. We also assayed KS tumors for LANA-NBs and whether they colocalized with factors identified in cell culture models. We found that LANA forms LANA-NBs in most, but not all LANA+ KS cells. However, DAXX which almost always colocalized with LANA-NBs in cell culture, rarely colocalized with LANA-NBs in KS tumor tissue. RNA-seq revealed the inducible nitric oxide (iNOS/NOS2) is highly upregulated in KS tumor tissue. We found that antibodies to iNOS reacted strongly to cells containing LANA-NBs, but, did not directly colocalize with LANA-NBs. Rather, iNOS tended to accumulate in perinuclear aggresomes or inflammasome-like structures in LANA+ KS cells. We also assayed KS lesions for the accumulation of nitrotyrosine, a modification associated with iNOS expression and stress relating to nitric oxide production. We found that antibody to nitrotyrosine stained large nuclear structures formed in KS lesions, but not in control skin samples. Nitrotyrosine containing bodies partially overlapped with LANA-NBs, suggesting that LANA and other proteins may be regulated by nitrotyrosine. Preliminary studies suggest iNOS and nitrotyrosine are weakly upregulated by KSHV in HUVEC cells. We speculate that iNOS induction in KS lesions leads to accumulation of nuclear proteins modified by nitrotyrosine, and that this may be an important and under-appreciated signaling pathway in KS.

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5.5

A panel of KSHV recombinant viruses targeting the polycistronic *Kaposin* locus for precise analysis of individual protein function

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KSHV replication is comprised of two phases: latent (minimal gene expression, absence of virion production) and lytic (full gene expression, active viral genome replication and progeny virion production). Despite the abundance of kaposin mRNA found in both latent and lytic phases of viral replication, the function of the *kaposin* locus during infection remains unclear. At least three proteins can be produced from the kaposin mRNA: Kaposin A (KapA), Kaposin B (KapB) and Kaposin C (KapC). Of these, we know the most about KapB, which we have showed in ectopic overexpression models is sufficient to induce EC spindling and stabilize AU-rich element (ARE)-containing proinflammatory mRNAs via the disassembly of cytoplasmic granules called processing bodies (PB), thereby contributing to the inflammatory nature of KS lesions. Here, we describe the construction of four different kaposin-deficient BAC16 viruses using strategies that either delete or recode kaposin ORFs individually or in combination: ΔKapABC, ΔKapBC, ΔKapB, ΔKapC and a partial revertant. We show that each can be used to establish latency in iSLKs and be reactivated into a full lytic replication cycle that produces infectious viral progeny for subsequent analysis. However, we show that despite several attempts to create a high genome-copy latent iSLK cell line containing BAC16ΔKapB, that this recombinant virus failed to establish robust latency, as evidenced by decreased viral genome copy number, small LANA nuclear bodies and decreased LANA protein level. Nevertheless, we were able to compare BAC16ΔKapB and wild-type BAC16 to demonstrate that following *de novo* infection of ECs, KapB is not required for EC spindling, but is necessary for PB disassembly. These findings reveal the utility of our suite of kaposin-deficient viruses which now permit the precise analysis of the roles of individual kaposin protein products during KSHV infection, making them of high value to the KSHV field.

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5.6

RYBP is Recruited to the KSHV Genome in PRC1-independent Manner and Represses Lytic Genes During Primary Infection

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Polycomb group (PcG) proteins are evolutionary conserved cellular transcription repressors that also play a significant role in the establishment and maintenance of KSHV latency. PcG proteins are known to form two different complexes: Polycomb Repressive Complex 1 and 2. PRC1 is usually recruited to its target promoters via binding to the repressive histone mark H3K27me3, which is deposited by PRC2. However, PRC1 has multiple forms that include both common and unique PcG proteins (non-canonical or ncPRC1), which can bind to and repress their target genes in a PRC2-independent manner. While PRC2 binds to the KSHV genome after 24 hpi, some PRC1 factors were shown to bind to KSHV lytic promoters prior to PRC2 suggesting that ncPRC1 can also control viral gene expression during *de novo* KSHV infection. To test the function of PRC1 factors in the establishment of KSHV latency, we performed an shRNA screen by targeting 8 different components of the canonical and non-canonical PRC1 during primary KSHV infection. We found that the shRNA knockdown of RYBP (RING1 and YY1 Binding Protein), a main subunit of ncPRC1, strongly induced viral lytic gene expression indicating that RYBP is involved in repression of viral lytic genes following primary KSHV infection. ChIP assays showed that RYBP is recruited to viral lytic promoters as early as 4 hpi. We demonstrated that the repressive function of RYBP on KSHV lytic genes is through the inhibition of the viral replication and transcription activator (RTA) expression. Furthermore, shRNA knockdown of PRC1 factors followed by ChIP experiments and analysis of RYBP mutants showed that the recruitment of RYBP to KSHV genome is PRC1-independent. Altogether, our data indicate that RYBP plays a crucial role in downregulating KSHV lytic genes following primary infection by repressing RTA expression thereby promoting the establishment of latency in a PRC1-independent manner.

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Poster Session I

Day 2, June 22nd

11:00am-1:00pm

P1.1

Anti-inflammatory agent dexamethasone suppresses KSHV-mediated oncogenesis by inducing interleukin-1 receptor antagonist

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Kaposi's sarcoma (KS), caused by Kaposi's sarcoma-associated herpesvirus (KSHV) infection, is the most common cancer in HIV-infected patients. KSHV-induced inflammation is a hallmark of KS. Interleukin-1 (IL-1) family represents a major mediator for inflammation and plays an essential role in both innate and adaptive immunity. Using a KS model of KSHV-induced cellular transformation, we screened for inhibitors that specifically inhibited KSHV-induced cell proliferation. Over half of the selected inhibitors had anti-inflammatory properties. One of these inhibitors is dexamethasone, a glucocorticoid receptor (GR) ligand and a commonly used anti-inflammatory corticosteroid. Treatment with dexamethasone inhibited cell proliferation, and colony formation in soft agar of KSHV-transformed cells but had a minimal effect on the matched primary cells. Dexamethasone induced cell cycle arrest but did not increase apoptotic cells of KSHV-transformed cells. RNA Seq analyses revealed that interleukin-1 receptor antagonist (IL-1RA) was downregulated by 26-fold in KSHV-transformed cells compared to primary cells; however, this was reversed by following dexamethasone treatment, suggesting that IL-1RA inhibition is essential for KSHV-induced cell proliferation and cellular transformation. Indeed, overexpression of IL-1RA in KSHV-transformed cells significantly inhibited cell proliferation and cellular transformation. Together, these results reveal the important role of IL-1 signaling in KSHV-induced oncogenesis, which can be inhibited by dexamethasone-induced anti-inflammatory GR signaling. Dexamethasone and likely other anti-inflammatory drugs could be promising therapeutic agents for KSHV-related cancers.

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P1.2

The cellular Notch1 Protein Promotes KSHV reactivation in a Rta-dependent manner

This poster has been moved to Poster Session 2 as P2.27.

P1.3

Incoming KSHV Factors Reprogram Oral Epithelial Cells during Infection

Natalie Atyeo[1], Seung Jin Jang[1], Min Chae[1], Aria Sharma[1], Katherine Glickman[1], Thomas Hong Nguyen[1], Zsolt Toth[1,2,3], **Bernadett Papp**[1,2,3,4,5]

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Kaposi's sarcoma-associated herpesvirus can replicate in oral epithelial cells, promoting viral transmission via saliva. Our transcriptome analysis of KSHV-infected primary human gingival epithelial (HGEPs) cells revealed that infection alters expression of over a thousand genes within 8 hours of infection. By comparing these KSHV infection-modulated genes with the genes, which are known to be induced by KSHV Virus-like Vesicles (VLVs), we found that the majority of the shared genes were related to epithelial differentiation, including master regulators of differentiation (PRDM1, FOXQ1) and the epithelial differentiation cluster (EDC) genes. Since VLVs and the triggered PRDM1 and epithelial differentiation can induce productive KSHV infection, we postulated that incoming KSHV proteins might be specifically needed to rapidly reprogram epithelial cells to promote KSHV lytic infection. In accordance, we found that lytic infection-defective KSHV failed to induce FOXQ1, PRDM1 and EDC genes in oral epithelial cells. To identify viral proteins that can induce these epithelial differentiation genes in HGEPs, we transduced HGEP cells with a lentiviral library of KSHV factors. Key lytic cycle promoting immediate-early proteins ORF45 and RTA were both sufficient to induce FOXQ1 in HGEP cells, but only ORF45 and early gene vIRF1 induced EDC genes, indicating a highly specialized function of incoming and rapidly expressed viral factors in triggering epithelial gene expression. Importantly, a point mutant of ORF45 lacking its lytic cycle-promoting RSK-signaling pathway sustaining function failed to induce FOXQ1 and EDC genes in oral epithelial cells. Furthermore, a vIRF1 mutant that has point mutation in its DNA-binding domain failed to induce the majority of vIRF1's host target genes genome-wide, including the EDC genes, showcasing the DNA-binding dependent activator function of vIRF1. In summary, we describe a positive feedback loop in which incoming herpesvirus proteins drive rapid reprogramming of oral epithelial cells to promote lytic KSHV infection in the oral cavity.

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P1.4

Uracil DNA Glycosylase of Gammaherpesvirus binds abasic site with high affinity and supports high-fidelity uracil repair with distinct motifs

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The gammaherpesviruses (gHV) which include human Epstein Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV) and murine gammaherpesvirus 68 (MHV68) are associated with B cell malignancy including Burkitt lymphoma. All herpesvirus encodes a viral homolog of the mammalian Uracil DNA-glycosylase (UNG) gene. In B cells, mammalian UNG plays a critical role in the antibody diversification processes of class switch recombination (CSR) and somatic hypermutation (SHM). UNG processes U:G mismatches created by activation-induced cytidine deaminase (AID) in an error-prone fashion to accomplish CSR and SHM. During primary infection, gHV expands in the germinal center B cells which actively express AID and undergo CSR and SHM. However, a role for viral UNG (vUNG) in pathogenesis and host genome integrity during the mutagenic germinal center reaction is not clear. To define differences between host and viral UNG repair function we used a cell based hypermutation reporter assay. We find that host UNG supports error-prone repair and mutagenesis while vUNG results in only high-fidelity error-free repair of AID induced lesions. Domain swap mutants suggest that the N-terminus of mammalian UNG is a determinant of mutagenic uracil processing. In vitro biochemical analysis finds several key differences between vUNG and host UNG, including the ability to bind abasic sites. Analysis demonstrates the vUNG extended leucin loop is a key structural determinant of these differences. These results define several key structural features that dictate divergent abilities of the UNGs in substrate and abasic binding, as well as downstream repair outcome.

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P1.5

Role of Kaposi Sarcoma-associated Herpesvirus Glycoproteins in Viral Infection

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Kaposi sarcoma-associated herpesvirus (KSHV), the causal agent of Kaposi sarcoma and two types of B-cell lymphoma, remains a significant public health burden. The development of an effective vaccine or therapeutic treatments against KSHV is limited by a poor understanding of how the virus initiates acute primary infection in diverse human cell types. The role of KSHV glycoproteins in virus entry mechanisms remains largely unresolved. Currently, it is postulated that KSHV entry involves initial binding of the viral glycoproteins ORF4 and K8.1 to the cellular receptor heparan sulfate (HS), concentrating the virus on the surface of the cell. Subsequently, viral protein–HS complexes trigger coordinated conformational changes in the core fusion/entry proteins gH/gL, thus facilitating gB fusion to initiate entry. However, this prevailing dogma is speculative, with no direct evidence of ORF4/K8.1 interacting with gH/gL or gB in a natural infection setting. To systematically characterize the roles of ORF4, K8.1, and gH in the viral life cycle and in determining cell tropism, we generated and characterized various KSHV mutants in which the expression of individual or multiple glycoproteins was abrogated. Using a bacterial artificial chromosome containing a complete recombinant KSHV genome and recombinant DNA technology, we inserted stop codons into the protein-coding regions. Using electron microscopy, we uncovered that the ORF4-, K8.1-, and gH-null viruses assembled and exited from cells normally compared to wild-type virus. Using purified virions, we assessed the infectivity of the mutants in diverse mammalian cell types *in vitro*. Where the gH-null viruses showed impaired infectivity, viruses lacking only ORF4 and/or K8.1 showed infectivity similar to wild-type virus in the epithelial, endothelial, and fibroblast cell types tested. Together, these results suggest that gH is indispensable for KSHV infection, while K8.1 and ORF4 are dispensable in the initiation of the KSHV entry mechanism in diverse human cell types.

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P1.6

Characterizing Expression and Regulation of Human and Viral Circular RNAs

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Multiple herpesviruses have been recently found to regulate human circular RNA (circRNAs) expressions and to encode viral circRNAs. Like cellular circular RNAs, these RNAs lack poly-A tails and their 5' and 3' ends have been joined, which confers protection from RNA exonucleases. The regulatory mechanism of circRNAs by viruses is largely unknown and viral circRNA expression profiles in vivo is limited. We first examined the expression patterns of circular RNAs from Kaposi's sarcoma herpesvirus (KSHV) in various environments. We performed deep sequencing of circRNA-enriched total RNA from a KSHV-positive patient lymph node for comparison with previous circRNA-Seq results. We found that circvIRF4 is highly expressed in the KSHV-positive patient sample relative to both B cell lines and de novo infected primary vascular and lymphatic endothelial cells. Overall, this patient sample showed a viral circRNA expression pattern more similar to the pattern from B cell lines, but we also discovered new back-spliced junctions and additional viral circular RNAs in this patient sample. We validated some of these back-spliced junctions as circular RNAs with RT-qPCR utilizing RNase R and divergent primers, and amplicon sequencings. Differential expression patterns of circular RNAs in different cell types led us to investigate what cellular factors might be influencing the ratio of viral linear mRNAs to circular RNAs. We found that repression of certain RNA-binding proteins shifted the balance between viral linear mRNAs and circular RNAs, suggesting possible mechanism by which KSHV regulate circRNA expression during infection. Taken together, examining viral circular RNA expression patterns may become useful tools for discovering their functions, the regulators of their expression, and determining the stage and cell types of infection in humans.

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P1.7

Phase I Study of Lenalidomide Combined with Dose-adjusted EPOCH and Rituximab (EPOCH-R^2) in Primary Effusion Lymphoma in Participants with or without HIV (NCT02911142)

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There is no standard therapy in primary effusion lymphoma (PEL), and prognosis is poor compared to other HIV-associated lymphomas with median survival of 10-22 months. In a prospective phase 1 study we evaluated the safety of etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone, and rituximab (EPOCH-R) with lenalidomide (EPOCH-R^2) in untreated PEL. Participants received EPOCH-R days 1-5 and lenalidomide days 1-10 starting at 25 mg (with dose de-escalation if toxicity occurred) every 21 days for 6 cycles. Participants with leptomeningeal PEL (CSF-PEL) received intrathecal chemotherapy; intrathecal prophylaxis was given to participants without CSF-PEL. All participants received thromboprophylaxis, opportunistic infection prophylaxis, and antiretroviral therapy (ART). Response to treatment, immune reconstitution, and overall survival (OS) were evaluated using descriptive statistics, Wilcoxon signed-rank test, and Kaplan-Meier methodology. 6 HIV$^+$ cisgender men with stage 4 PEL were enrolled July 2017-August 2019. 3 had CSF-PEL and 1 had bone marrow involvement. 4 had Kaposi sarcoma; 1 also had multicentric Castleman disease. Median CD4$^+$ was 231 cells/µL (IQR: 10, 310) at baseline and 189.5 cell/µL (IQR: 56, 224) at end-of-treatment, which was not a significant decline (p=0.46). The most common adverse events graded by CTCAEv5 were hematologic, including grade 4 neutropenia (100%), leukopenia (100%), thrombocytopenia (67%) and CD4$^+$ lymphopenia (67%). 3 participants developed pulmonary emboli despite thromboprophylaxis. No participants developed opportunistic infections. There were no dose-limiting toxicities; lenalidomide 25 mg is the recommended phase 2 dose. 1 participant with progressive disease after 5 cycles went on to receive additional therapy. 1 participant died of progressive disease, and 1 died of HIV-related complications without PEL at autopsy. The complete response rate was 50% (95%CI: 11.8-88.1). 2-year overall survival was 66.7% (95% CI:19.5-90.4), and median OS was not reached. Front-line PEL treatment with EPOCH-R^2 is safe. The most common toxicities were hematologic with preliminary evidence of activity and good OS.

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P1.8

IKKalpha-Mediated Non-Canonical NF-kappaB Signaling is Required to Support Murine Gammaherpesvirus 68 Latency In Vivo

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Non-canonical NF-kappaB signaling is activated in B cells via TNF receptor superfamily members such as the Lymphotoxin beta-R and BAFF-R. The non-canonical pathway is required at multiple stages of B-cell maturation and differentiation, including the germinal center reaction. However, the role of this pathway in gammaherpesvirus latency is understudied in vivo. Murine gammaherpesvirus 68 (MHV68) is a genetically tractable system used to define pathogenic determinants. Mice lacking the BAFF-R exhibit defects in splenic follicle formation and are greatly reduced for MHV68 latency. Here, we describe a novel approach to disrupt non-canonical signaling exclusively in cells infected with MHV68. We engineered a recombinant virus that expresses a dominant negative form of IKKalpha, named IKKaSA, with S176A and S180A mutations that prevent phosphorylation by the upstream NIK kinase. We controlled for the transgene insertion by introducing two all-frame stop codons into the IKKaSA gene. The IKKaSA mutant but not the IKKaSA.stop control virus impaired LTbetaR-mediated activation of the NF-kappaB p52 subunit in primary fibroblasts. The IKKaSA mutant had no replication defect in primary fibroblasts in culture or in the lungs of mice following intranasal inoculation. However, the IKKaSA mutant was severely defective in colonization of the spleen and in the establishment of latency (~1/27,295) compared to the IKKaSA.stop control (~1/100) and WT MHV68 (~1/100) at 16 dpi. Reactivation was undetectable in splenocytes infected with the IKKaSA mutant. In contrast, the frequency of virus latency and reactivation within the peritoneal compartment did not differ between IKKaSA and IKKaSA.stop infections. Taken together, the non-canonical NF-kappaB signaling pathway is essential for the establishment of latency in the secondary lymphoid tissue of mice infected with the murine gammaherpesvirus pathogen MHV68. Antagonizing cytokine engagement with B cells that drives signaling through this pathway may provide a novel therapeutic avenue to combat gammaherpesvirus latency and lymphoproliferation.

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P1.9

Regulation of LANA expression through the interaction of nucleolin with g-quadruplexes in the mRNA

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Kaposi's sarcoma associated herpesvirus (KSHV) is linked to several human malignancies. KSHV establishes latent life-long infection during which it evades the host's immune surveillance, while maintaining the viral genome through latent DNA replication. Latency associated nuclear antigen (LANA) is primarily expressed during this time and is responsible for the replication and maintenance of the viral genome. Replication of the viral genome can be regulated by various factors including secondary structures in the genomic sequences. G-quadruplexes (G4s) are highly stable secondary structures, which can control various cellular processes including replication and transcription. We previously showed that the stabilization of G-quadruplexes in guanine rich nucleic acid sequences, altered replication, initiation, and replication fork movement. We previously identified multiple G4 sites in LANA mRNA, which plays a major role in regulating the levels of LANA in infected cells. These G4 sites interact with cellular proteins to stabilize/destabilize mRNA in order to regulate LANA translation. Specifically, our data shows that G-quadruplexes can be unfolded by hnRNP A1, which results in increased translation of LANA protein. Stabilization of G-quadruplexes with ligands led to a decrease in the LANA translation. Expression of EBNA1, an ortholog of KSHV LANA of Epstein-Bar Virus (EBV), has been shown to be regulated by G-quadruplexes through its interaction with Nucleolin (NCL), which is a multifunctional DNA/RNA binding protein. We explored the role of NCL in regulating LANA expression through its interaction with the G-quadruplexes in the mRNA of LANA. Using an array of biophysical and biochemical assays, we demonstrated that NCL regulates LANA through G-quadruplex stabilization. In turn, this results in the downregulation of LANA's translation and reduction in antigen presentation on the cell surface. G-quadruplexes provides a therapeutic avenue for controlling KSHV latency.

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P1.10

A Pentameric Protein Ring with Novel Architecture Is Required for Herpesviral Packaging

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Packaging of the herpesviral genome into nascent capsids is a process that is thought to be mechanistically similar to that of the tailed bacteriophages. It depends on an ATP-dependent packaging motor called the terminase, a portal protein through which the genome is threaded, and capsid proteins to contain the genome. Cleavage of the DNA into a unit-length genome by the terminase occurs concomitantly with packaging, and failure to package renders the entire viral amplification process null. Herpesviruses contain an additional conserved protein not present in bacteriophage that is essential for packaging; however, the function of this protein is unknown. We present structures of this protein from Kaposi's sarcoma-associated herpesvirus (KSHV ORF68) and Epstein Barr Virus (EBV BFLF1). The structures reveal that both proteins form homopentameric rings where each monomer is stabilized by multiple zinc finger motifs. Negative stain EM of UL32, the homolog from HSV-1, suggests that it similarly forms a pentameric ring and that the architecture of the complex is conserved across the herpesviruses. We previously observed that ORF68 binds nucleic acid in vitro. Our structure of ORF68 revealed that the central channel of the ring is positively charged, suggesting a potential role in nucleic acid binding. Indeed, mutation of individual positively charged residues within but not outside the channel ablates DNA binding, and in the context of KSHV infection these mutants fail to package the viral genome or produce progeny virions. We propose a model in which ORF68 facilitates the transfer of newly replicated viral genomes to the packaging motor, the first potential role for this enigmatic packaging accessory factor.

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P1.11

Pomalidomide-Mediated Caspase Cleavage of K3 and K5 Prevents MHC-I Downregulation in Lytic Primary Effusion Lymphoma Cells

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Pomalidomide (Pom) is effective in the treatment of multiple myeloma and was recently approved for treatment of Kaposi's sarcoma. Virus-infected cells evade immune detection by down-regulating surface immune markers, and this can thwart immune based therapy for virus-induced tumors. We previously showed that Pom can prevent lytic induced down-regulation of MHC-I in primary effusion lymphoma (PEL) cells. Here, we describe one mechanism by which Pom prevents downregulation of MHC-I. Downregulation of MHC-I during lytic replication occurs largely through the action of viral E-3 ubiquitin ligases, K3 and K5, which ubiquitinate a number of immune surface markers upon lytic activation, leading to their destruction. Based on immunoblot analysis, we found that Pom treatment of lytic PEL cells decreased the levels of K3 and K5 by inducing caspase cleavage of these proteins. Based on both immunoblot and FACS analysis, ZVAD, a pan caspase inhibitor, was able to block caspase cleavage leading to restoration of K3 and K5 protein levels and partial restoration of lytic induced downregulation of MHC-I. To verify caspase cleavage of K5, we expressed GFP-K5 in BJAB cells and treated protein extracts with different caspases. C-terminal cleavage of GFP-K5 was observed with caspases 3 and 8 but not caspase-1. Using RP-HPLC/MS TOF analysis, we identified a caspase cleavage site in the C-terminal tail of K5. Our data indicate that Pom-mediated caspase cleavage of K3 and K5 interferes with MHC-I downregulation by these viral proteins. These studies reveal a novel mechanism by which Pom prevents the MHC-I downregulation in KSHV infected PEL cells during lytic replication that in turn leads to increased immune recognition of these complex viral-malignancies.

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P1.12

Targeting MYC Transcription with Small Peptide Derived from KSHV Transactivator

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Like all other viruses, KSHV relies on host cell transcription and translation machineries for replication. KSHV proteins thus function to redirect multiple cellular proteins for viral replication. In KSHV replicating cells, host cell gene transcription is frequently down-regulated because important transcriptional apparatuses are appropriated by viral transcription factors. Here we show that an evolutionally-shaped viral protein sequence is a great starting material for unique drug development to modulate cellular transcription. Cellular c-Myc protein (MYC) is overexpressed in over 70% of all types of cancer cells and therefore a very attractive target to control cancer cell growth. We identified a small functional peptide (named VGN50: Virus *de* Gann *wo* Naosu ORF50) from the K-Rta intrinsically-disordered transactivation domain, which strongly attenuates MYC expression, reduces cell proliferation, and selectively kills cancer cells in both tissue culture and a xenograft tumor mouse model. Target gene profiling with thiol (**S**H)-**l**inked **a**lkylation for the **m**etabolic sequencing (SLAM seq) clearly demonstrated that the VGN50 targets MYC expression with the highest an FDR-adjusted p-values of 9.51 E-12 among all 25,000+ cellular genes in BCBL-1, and Gene Sets Enrichment Analyses showed IFN pathway is the primary target of the peptide. Treatment with the VGN50 in xenograft PEL also demonstrated attenuation of inflammatory cytokine production from PEL cells in ascites fluids. Mechanistically, VGN50 blocks recruitment of coactivator complex, which consists of Nuclear receptor coactivator 2, p300, and SWI/SNF to MYC promoter in leukemia cells. Furthermore, fusing the 13 amino acids peptide with humanized anti-CD22 single chain armed the antibody drug with cell killing ability, and inhibited SU-DHL-10 cell growth in soft agar. Our studies thus demonstrated the utility of the peptide sequence as a therapeutics module, which may be used to regulate MYC expression in a cell type specific manner by fusing with targeting ligands.

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P1.13

Regulation of Immune Surface Molecules by CDK4/6 Inhibitors in Gammaherpesvirus-infected Tumor Cells

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Cyclin dependent kinase 4 and 6 (CDK4/6) inhibitors have been approved for certain breast cancers. KSHV, the cause of primary effusion lymphoma (PEL) and Kaposi sarcoma (KS), downregulates immune surface molecules, such as MHC-I, ICAM-I, and B7-2; this enables KSHV to evade T-cell and natural killer (NK) cell immunity. Goel et al recently reported that CDK4/6 inhibition enhances expression of MHC-1 in murine models of breast cancer (*Nature*, 2017, doi 10.1038/nature23465) and Manzano et al. reported that cyclin D2 is required for survival of PEL cells (*Nat. Communcations*, 2018, doi 10.1038/s41467-018-05506-9). Here, we show that the three FDA approved CDK4/6 inhibitors, abemaciclib, palbociclib, and ribociclib, inhibit cell growth in both PEL lines and KSHV-infected human umbilical vein endothelial cells (HUVEC). A similar inhibitory effect was observed for EBV+ Burkitt's lymphoma (BL) cell lines. Pretreatment with CDK4/6 inhibitors variously increased MHC-I surface expression during latency in all cell lines tested. It also prevented the downregulation of MHC-I surface expression during lytic replication in KSHV-infected cells. Also, CDK4/6 inhibitors variably increased surface expression of ICAM-1, B7-2, and PD-L1 in all cell lines tested at clinically achievable concentrations. Moreover, treatment of PEL and BL cells with abemaciclib significantly enhanced T-cell activation. Together, these observations suggest that the CDK4/6 inhibitors can potentially thwart virus-induced immune evasion and cytotoxic T cell killing of the tumors. Transcription analysis showed that viral genes were differentially increased by CDK4/6 inhibitors in most PEL and BL lines. Also, expression of endogenous retrovirus ERV3-1 was increased, possibly due to inhibition of DNA methyltransferase expression. It is possible that the enhancement of cell surface markers results from increase in interfgeron-stimulated genes in response to the viral upregulation. These results provide a rational for the clinical testing of these drugs in KSHV-induced tumors.

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P1.14

Characterizing Cellular Mutations in Kaposi Sarcoma Tumor

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OBJECTIVE: Kaposi sarcoma (KS) is considered an AIDS-defining malignancy; however, it is unclear whether KS is primarily a virally-driven neoplasm or if cellular genetic alterations drive cellular proliferation in some cases. Despite the large number of studies that have focused on understanding Kaposi sarcoma-associated herpesvirus (KSHV) and viral mechanisms of pathogenesis, the cellular genome of KS has not been systematically studied and remains largely unexplored. **METHODS**: We performed whole exome sequencing on KS tumors and matched normal control skin from adults with HIV-associated KS receiving treatment at the Uganda Cancer Institute in Kampala, Uganda. Samples were mapped and analyzed using computational pipelines for DNA alterations. **RESULTS:** 79 KS tumors and 56 matched normal skin samples have been sequenced to date. Based on preliminary analysis of 31 KS tumor-normal skin pairs, somatic cellular genetic alterations were present in all tumors, although the mutational burden was relatively low in most tumors. Recurrent mutations were observed in 2 or more samples, including mutations in potentially functionally relevant genes, but no mutations in a single pathway were consistently altered. Based on variant allele frequency (VAF), all tumors appeared to contain multiple independent clones with different alterations, and approximately one-third of cases contained VAF clusters indicating expansion of specific clonal populations. **CONCLUSIONS:** These preliminary findings indicate that KS tumors carry a range of somatic cellular mutations, which may help explain the histologic and clinical heterogeneity of KS. The low mutational burden observed in a majority of samples suggests KSHV latent gene expression is likely more important than clonal transformation in driving tumorigenesis. However, clonal populations are evident in some tumors, indicating that KS may exist as a range of polyclonal to monoclonal tumors. Mutations observed in functionally relevant genes may also alter cellular pathways and contribute to tumor development.

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P1.15

Inhibition of the eIF4E-kinase MNK as a Strategy for Controlling Kaposi's Sarcoma-Associated Herpesvirus Replication

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During reactivation, KSHV hijacks the host cap-dependent translation machinery to produce the numerous viral proteins required for subsequent infection. Cap-dependent translation relies on many different pathways to induce phosphorylation events that modulate the assembly and activity of the translation initiation complex. Two such pathways, p38 and ERK, converge on the phosphorylation of MAP kinase interacting kinase 1 and 2 (Mnk1 and Mnk2). Mnk1/2 are the sole kinases for the cap-binding protein eIF4E. The phosphorylation of eIF4E is associated with oncogenesis, and our previous observations indicate that Mnk1/2 phosphorylate eIF4E during the KSHV lytic cycle. Our goal is to define how eIF4E phosphorylation impinges on KSHV infectivity. Treatment of KSHV infected cells with the Mnk1/Mnk2 small-molecule inhibitor Tomivosertib results in a profound reduction in the translation of viral proteins and overall lytic replication without deleterious impact on the host cell. These observations suggest a requirement for eIF4E phosphorylation during the lytic cycle of KSHV. To further dissect the contribution of Mnk1 and Mnk2 to eIF4E phosphorylation during KSHV replication, we have developed single and double Mnk1/2 knockdown cell lines. We are currently evaluating the impact of the ablation of these kinases on KSHV reactivation. Our results will provide valuable information on the potential of Mnk1 and Mnk2 as therapeutic targets for the treatment of KSHV-associated malignancies.

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P1.16

Prox1-induced Lytic replication of Kaposi sarcoma herpes virus promotes Kaposi sarcoma development

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Kaposi sarcoma is the most common cancer in human immunodeficiency virus–positive individuals and is caused by Kaposi sarcoma–associated herpesvirus (KSHV). It is believed that a small number of latently infected Kaposi sarcoma tumor cells undergo spontaneous lytic reactivation to produce viral progeny for infection of new cells. Here, we use matched donor-derived human dermal blood and lymphatic endothelial cells (BEC and LEC, respectively) to show that KSHV-infected BECs progressively lose viral genome as they proliferate. In sharp contrast, KSHV-infected LECs predominantly entered lytic replication, underwent cell lysis, and released new virus. Continuous lytic cell lysis and de novo infection allowed LEC culture to remain infected for a prolonged time. Because of the strong propensity of LECs toward lytic replication, LECs maintained virus as a population, despite the death of individual host cells from lytic lysis. The master regulator of lymphatic development, Prox1, bound the promoter of the RTA gene to upregulate its expression and physically interacted with RTA protein to coregulate lytic genes. Thus, LECs may serve as a proficient viral reservoir that provides viral progeny for continuous de novo infection of tumor origin cells, and potentially BECs and mesenchymal stem cells, which give rise to Kaposi sarcoma tumors. Our study reveals drastically different host cell behaviors between BEC and LEC and defines the underlying mechanisms of the lymphatic cell environment supporting persistent infection in Kaposi sarcoma tumors.

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P1.17

ORF45 is a novel inhibitor of p53-signalling encoded by Kaposi Sarcoma-associated Herpesvirus

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Kaposi Sarcoma-associated Herpesvirus (KSHV) or Human Herpesvirys Herpesvirus 8 (HHV8) is a carcinogenic dsDNA virus and an etiological agent of Kaposi's Sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman's Disease (MCD). In order to prevent early apoptosis and support its replication and proliferation, the virus expresses series of open reading frames (ORF) that tightly control and regulate p53 expression: latency-associated nuclear antigen (LANA1), viral interferon regulatory factor -1, -3, and-4. Here we describe a novel inhibitor of p53 signaling encoded by ORF45 and identify the mechanisms of its action: (i) ORF45 binds p53 and (ii) prevents its interactions with USP7, a p53 deubiquitinase thereby decreasing its accumulation, (iii) mislocalizes p53 to the cytoplasm, and (iv) blocks its transcriptional activity.

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P1.18

A KSHV microRNA Downregulates Anti-Angiogenic Cellular lncRNA MEG3 in Endothelial Cells

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The Kaposi's Sarcoma-Associated Herpesvirus (KSHV)-encoded microRNAs (miRNAs) are expressed during viral latency and have been shown to contribute to a number of processes associated with oncogenesis. Although miRNAs normally perform their function of post-transcriptional gene repression in the cytoplasm, the necessary components for this process are in the nucleus as well. This means that there is opportunity for miRNAs to interact with long noncoding RNAs (lncRNAs), which are also found in the nucleus. A lncRNA is any RNA greater than 200 nt that does not code for protein. Given this broad definition, there is a substantial diversity of function within this group. Several of the better-studied lncRNAs are known to have roles in cancer. Recently, we performed RNA-seq analysis on Telomerase-Immortalized Vein Endothelial (TIVE) cells latently infected with a panel of KSHV miRNA knockout viruses. Each mutant virus lacks one of the twelve pre-miRNAs encoded by KSHV. We found that many of the genes which were differentially expressed between wt KSHV and the mutant viruses were lncRNAs. This is indicative of frequent interaction between the KSHV miRNAs and host lncRNAs during viral latency. Our analysis showed that one of the most differentially expressed lncRNAs in several of the mutants was Maternally Expressed 3 (MEG3). MEG3 is a well-known tumor suppressor that inhibits cell proliferation and angiogenesis. Interestingly, we had previously identified MEG3 as a target of the KSHV miRNAs through microarray analysis. At that time, we were able to show that MEG3 specifically interacts with KSHV miR-K12-6-5p and can be downregulated by a mimic of this miRNA[1]. Study of additional KSHV miRNA/lncRNA pairs identified through RNA-seq will help to clarify the relationship between lncRNAs, miRNAs, and KSHV-driven oncogenesis.

[1]Sethuraman et al., PLoS Pathog, 2017.

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P1.19

High KSHV seroprevalence among MSM Persons with HIV (PWH) in an urban safety-net health center in the southern United States; evidence of racial/ethnic disparities

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Despite a decrease in Kaposi's sarcoma (KS) cases in much of the US, the incidence of KS and associated mortality is increasing in specific subpopulations, particularly young, African American men in the South. To further understand this disparity, we sought to describe the seroprevalence and risk factors associated with Kaposi's sarcoma herpesvirus (KSHV) among men who have sex with men (MSM) and transgender women (TGW) with HIV in Dallas, Texas. We enrolled MSM and TGW with HIV from a large urban safety-net clinic in Dallas. Blood samples were collected from participants for IgG testing (K8.1 and ORF73), followed by KSHV PCR on blood and saliva samples for those with positive IgG results. We also collected demographics, sexual history, sexual practices, HIV history, substance use, and insurance status. Of 107 participants, 65 (60.7%) were seropositive for KSHV. Seroprevalence varied by race/ethnicity, with 18/21 (85.7%) Hispanic, 14/21 (66.7%) white, and 32/63 (50.8%) black participants testing positive for either one or both KSHV antibodies. Other risk factors associated with KSHV seropositivity include younger age, and 4 or more sexual partners in the past year. Current CD4 count, HIV viral load, history of sexually transmitted infections, tobacco use and recreational drug use were not associated with KSHV seropositivity. In conclusion, we found that approximately two-thirds of MSM and TGW with HIV in Dallas were KSHV seropositive, which is relatively high compared to other studies of US MSM with HIV (30-70%). In our study, KSHV was more common among Hispanic and white individuals, and was associated with younger age and increased numbers of sexual partners. Differences in KSHV seroprevalence alone are unlikely to explain racial disparities in the incidence of KS. Further study is needed to better understand drivers of KSHV infection and KSHV-related diseases in highly impacted groups in the US.

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P1.20

Evidence for multiple subpopulations of gamma herpesvirus-latently infected cells

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Kaposi Sarcoma-associated herpesvirus (KSHV)-associated primary effusion lymphoma (PEL) are traditionally viewed as homogenous regarding viral transcription and lineage of origin, but so far this contention has not been explored at the single cell level. Single cell RNA sequencing of latently infected PEL supports the existence of multiple sub-populations even within a single cell line. At most 1% of the cells showed evidence of near complete lytic transcription. The majority of cells only expressed the canonical viral latent transcripts: those originating from the latency locus, the viral interferon regulatory factor locus and the viral lncRNA "PAN"; however, a significant fraction of cells showed varying degrees of more permissive transcription; some showed no evidence of KSHV transcripts whatsoever. Levels of viral IL-6 / K2 mRNA emerged as the most distinguishing feature to subset KSHV-latently infected PEL. One newly uncovered phenotype is the existence of adherent BCBL-1 cells that readily adhered to fibronectin, and that displayed mesenchymal-lineage like characteristics.

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P1.21

Systematic Analysis of KSHV Genome Sequences from a Cameroonian Kaposi's sarcoma Case-Control Study finds no Association with Disease Risk

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Introduction: In sub-Saharan Africa, where Kaposi's sarcoma-associated herpesvirus (KSHV) is endemic, Kaposi's sarcoma (KS) is a significant public health problem. Until recently, KSHV genetic analysis was performed on variable gene regions representing only a fraction of the total genome. The possible contribution of sequence variations across the entire genome to disease pathogenesis is understudied. **Objectives**: This study is the first to systematically survey near full-length KSHV genome sequences in KS cases and controls to describe genome variability and to ascertain if sequence variation is significantly associated with disease risk. **Materials and Methods**: Forty-three samples with moderate to high KSHV load were obtained from a large KS case-control study nested within the IeDEA Central Africa cohort. Samples were sequenced using the SureSelect^{XT} target enrichment system for Illumina. **Results**: Near full length KSHV genomes were obtained from 20 KS patients and 23 control participants. Phylogenetic analysis of the K1 gene region indicated most strains were A5 or B1 subtypes, commonly observed in Africa. We identified evidence of recombination and unique polymorphisms throughout the genome, including complete and partial deletions of viral genes of significant functional importance. Further, dual infection with different KSHV strains was observed in three participants. A detailed analysis of KSHV genomes from KS patients and control participants did not find viral sequence variations associated with KS. **Conclusions**: Study strengths include direct comparisons of near full-length KSHV genomes from KS patients and control participants; no association between sequence variation and disease risk was observed. This study provides an additional 43 near full length KSHV sequences from a KSHV endemic area, describing unique sequence variants in the central "constant" regions of the genome, and highlights important sequence characteristics of strains circulating in sub-Saharan Africa. Finally, the study describes cases of coinfection by multiple strains and provides evidence for recombination.

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P1.22

WT1 Tumor Antigen is Overexpressed in Kaposi Sarcoma and is Regulated by KSHV vFLIP

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Background: Wilms' tumor 1 (WT1) is overexpressed and associated with poor prognosis in several hematologic and solid malignancies and has shown promise as an immunotherapeutic target. We evaluated WT1 expression in Kaposi sarcoma (KS), the most common HIV-associated malignancy, which is caused by the KS herpesvirus (KSHV or HHV-8). We assessed WT1 expression in KS in a cohort of >300 individuals with HIV-associated KS, and determined if KSHV infection accounts for this upregulation. We focused on vFLIP because of its ability to induce NF-kB, known to affect WT1 levels. **Methods:** We used immunohistochemistry to evaluate 303 biopsies of advanced HIV-associated KS from clinical trial AMC-066/A5263 (NCT01435018) for expression of WT1, LANA, and the presence of CD4+ and CD8+ T cells. Effects of KSHV on WT1 expression were examined *in vitro* using endothelial cell infection models to determine whether KSHV latent viral vFLIP (viral FLICE inhibitory protein) influences WT1 expression. A T cell receptor mimic antibody, ESK-1, specific for WT1 peptide/ HLA-A02 expression, was tested for its ability to bind KSHV-infected or vFLIP-expressing endothelial cells. **Results:** Moderate to strong WT1 expression was demonstrated in 65% of the 303 KS biopsies, and in 92% of nodular lesions. WT1 expression was positively correlated with increased histopathologic stage and expression of the viral latent oncoprotein (LANA; r=0.687, p=0.0001), and was inversely correlated with the quantity of CD8+ T cells (r=-0.2536 p=0.0001). *In vitro* infection with KSHV or vFLIP induction in endothelial cells resulted in WT1 upregulation and increased binding of ESK-1. **Conclusions:** WT1 is overexpressed in KS and is upregulated by vFLIP. Our data demonstrate increased binding of WT1 overexpressing endothelial cells by ESK-1. Immunotherapy directed towards WT1 may represent a new treatment strategy in KS

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P1.23

Elucidation of Biomarkers from Kaposi's Sarcoma Transcriptomics and Validation in Tumor Tissues

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Despite increasing use of anti-retroviral therapy and the resulting HIV suppression, HIV-associated epidemic Kaposi's sarcoma (EpKS) remains one of the most prevalent cancers in sub-Saharan Africa. Our lab previously reported differential gene expression profiles of EpKS tumors versus normal skin by comparative transcriptomics. These data revealed increased expression of common immune signaling cytokines such as CXCL9, CXCL10, and CXCL11 as well as dysregulation is genes associated with glucose and lipid metabolism. Our objective here was to leverage that human KS tumor transcriptome data to define protein biomarkers that are enriched in the EpKS tumor, low/absent in healthy tissues, and expressed on the cell surface. Such biomarkers could serve as candidate targets for therapeutics. By triaging our differential transcriptomic data using SURFY and other cell surfacesome algorithms, we identified a list of potential EpKS surface markers whose expression was also highly concordant with expression of KSHV latency locus genes. Cellular transcripts within the vascular endothelial growth factor (VEGF) signal transduction family and angiogenesis pathways were upregulated in EpKS tumor tissues as expected. Specifically, KDR, FLT4 and NRP2 were upregulated in EpKS tumors; all three of which are receptors in the VEGF pathway. Other specific cell surface markers included the netrin-1 axon receptor UNC5A, and the metalloprotease ADAM12. A subset of these markers was comparatively evaluated in KSHV infected L1T2 cells versus uninfected TIVE precursors. Additionally, L1T2 cells were implanted into SCID mice to induce KS-like tumors. Tumors derived from xenotransplanted mice exhibited similar biomarker staining patterns to human KS tissue. This suggests that L1T2 explant tumors could serve as an *in vivo* KS tumor model to characterize additional biomarkers and screen potential therapeutic agents against them. We are currently discriminating between KSHV infection-specific cell surface markers and those up-regulated in the tumor stromal support cells using dual-color immunohistochemistry and immunofluorescence.

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P1.24

Gene Expression Patterns in Skin and Gastrointestinal Kaposi Sarcoma Lesions

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Kaposi sarcoma (KS), caused by Kaposi sarcoma herpesvirus (KSHV), is a multicentric tumor characterized by abnormal vasculature and proliferation of KSHV-infected spindle cells. KS involves the skin but can also affect the gastrointestinal tract (GI) in severe cases. Here, we performed RNA sequencing of skin and GI KS lesions from patients with KS to understand the similarities and differences in the gene expression pattern. We obtained skin and GI KS lesions with matched normal skin and GI samples. Differential gene expression was measured by comparing KS lesions to normal matched samples. Twenty-five paired samples were obtained (skin (10 pairs) and GI (15 pairs)) from 23 patients with KS (21 patients had concurrent HIV infection). All tumors were stage T1. Twelve paired samples were from patients who had received prior KS therapy. In skin KS, cellular gene networks associated with cell adhesion (extracellular matrix), immune response, angiogenesis, and hypoxia were dysregulated when compared with normal skin. Among samples with higher KSHV sequencing reads, there were 68 human genes increased (2 decreased) in both skin and GI KS lesions. Notably, one of the genes that was upregulated included *FLT4*, which encodes for a receptor of VEGF-C and VEGF-D. There were more KSHV lytic viral genes detected in GI KS as compared to skin KS, which may be due to more advanced KS or a difference in lytic activation in GI tissues. Five patients had both skin and GI KS (with matched normal samples), which demonstrated that specific genes were strongly increased in both tissues. This is one of the first studies comparing skin and GI KS that highlights differences in viral gene and clinically relevant host gene expression between these tissues. By analyzing these gene expression patterns, this ongoing study will improve our understanding of KS pathogenesis.

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P1.25

Maternal Kaposi's sarcoma herpesvirus (KSHV) Antibodies and Risk of KSHV Seroconversion in a Cohort of Kenyan Children

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Infection with Kaposi's sarcoma herpesvirus (KSHV) in sub-Saharan Africa occurs primarily during childhood, with earlier infection linked to higher risk of Kaposi's sarcoma development. To determine whether transplacental transfer of anti-KSHV antibodies at delivery was associated with KSHV seroconversion before two years of age, mother-child pairs from a cohort study in Western Kenya that delivered at the study hospital were enrolled. Maternal venous blood and cord blood samples were collected at delivery and tested for anti-KSHV antibody levels to K8.1 and ORF73 by bead-based multiplex assay. Transplacental transfer of anti-KSHV antibodies was measured as cord-to-maternal ratio (CMR) levels and categorized into tertiles (low, medium, high). Children delivered at the study hospital had a venous blood draw at 12, 18, and 24 months of age. Children were considered KSHV seropositive if antibodies to K8.1 and/or ORF73 were detected in samples taken at any timepoint as tested by ELISA. The association between CMR anti-KSHV antibody levels and KSHV seropositivity by 24 months of age was modeled using chi-squared. 161 children were born at the study hospital and had at least one sample tested for KSHV at 12, 18, or 24 months of age. Child KSHV seropositivity was 17.4% at 12 months, 28.0% at 18 months, and 36.0% at 24 months. 62/161 children had maternal and cord blood samples tested by multiplex. There was no statistically significant association between KSHV seropositivity by age 24 months and CMR anti-K8.1 antibody (p=0.52) or anti-ORF73 antibody tertiles (p=0.56). Our findings suggest that transplacental transfer of anti-KSHV antibodies at delivery does not affect risk of KSHV seroconversion in infants under two. However, KSHV seroconversion in Kenyan infants by age two was high. To reduce risk of infection and disease, the factors leading to earlier KSHV seroconversion must be elucidated.

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P1.26

Daratumumab as a Potential Treatment for Primary Effusion Lymphoma

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Daratumumab (Dara) is an anti-CD38 monoclonal antibody that is FDA-approved for multiple myeloma (MM) and has shown clinical activity against NK T cell lymphoma. Dara mediates direct cytotoxicity primarily through Fc-related functions such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). Primary effusion lymphoma (PEL), a rare but aggressive non-Hodgkin's lymphoma caused by KSHV with a poor survival, generally does not express CD20 (the target for rituximab) but has variable expression of CD38. We performed pre-clinical evaluation of the ability of Dara to induce CDC and ADCC in PEL cell lines. We tested five PEL cell lines and observed that all of them express CD38, albeit at varying levels (BCBL-1, BC-1, BC-3 expressing high levels; BC-2 and JSC-1 expressing low levels). None showed an induction of CDC upon Dara-treatment. By contrast, Dara readily induced CDC in Daudi (a Burkitt's lymphoma line). The PEL lines were found to have high expression of CD59, a complement inhibitory protein, compared to Daudi, and this may account for the lack of CDC in PEL. Assessment of DARA-induced ADCC against the PEL cell lines was performed using Jurkat-NFAT cells (which express Fc-receptor and produce luciferase upon NFAT activation as a marker of ADCC induction) as the effector cells. Dara led to >4-fold induction of ADCC in high-CD38 PEL lines and only a minor induction (~2 fold) in low-CD38 lines. All trans-retinoic acid (ATRA), another FDA-approved drug, can increase CD38 levels in MM. We found that ATRA raises CD38 levels on the PEL cell surface as well, and this in turn increased Dara-induced ADCC of the low-CD38 expressing PEL lines. Taken together, these data suggest that Dara can induce ADCC of PEL, and is worth testing in PEL, possibly in combination with ATRA.

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P1.27

Kaposi's sarcoma-associated herpesvirus transcription regulation mechanism through interactions with non-coding (nc) 7SK RNA

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KSHV is an obligate intracellular parasite that relies on the host cell to provide components for replication. After an initial infection, latency is established, and the viral genome persists as an episome within host cells with periodic episodes of reactivation. During lytic reactivation, one of the viral factors expressed is the mRNA transcript accumulation (MTA) protein, open reading frame 57 (ORF57). ORF57 is a multifunctional regulator involved in facilitating lytic gene expression, primarily by enhancing the stability of intronless viral RNA and to promote efficient translation of viral transcripts. Additionally, ORF57 has been implicated in interacting with cellular and viral non-coding RNAs including spliceosome U1 and viral PAN RNA. We have preliminary data demonstrating a specific interaction between ORF57 and cellular ncRNA 7SK. 7SK plays a highly significant role in regulating transcription by modulating the activity of the Positive Transcription Elongation Factor-b (P-TEFb) and is involved in the regulation of RNA polymerase II elongation. Understanding the molecular basis of KSHV transcriptional regulation by ORF57 through the use of cellular components is critically important. To characterize this interaction, we performed RNA-IP with ORF57 and subsequent qPCR to quantify levels of associated transcripts in reactivated iSLK BAC16 cells and cells transfected with ORF57 expression plasmid. Furthermore, we performed Co-IPs and proteomic analysis that demonstrated ORF57 is not interacting with the proteins in the P-TEFb complex. The interaction between ORF57 and 7SK may be facilitated by K-bZIP, a viral protein reported to interact with ORF57 and 7SK. Thus, we hypothesize that ORF57 promotes viral transcription by interacting with 7SK RNA along with other viral proteins, resulting in the release of the P-TEFb, and an increase in RNA Pol II activity at viral promoters.

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P1.28

Epigenetic reprograming of KSHV genome during hypoxic reactivation

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Epigenetic reprogramming of KSHV genome in infected cells is essential for achieving latency through control of viral gene expression. Unwinding of these Epigenetic changes is considered necessary for reversing the process of virus latency to reactivate and drive productive replication. The reversal of these epigenetic changes under physiologically allowed conditions such as hypoxia has never been studied in KSHV infected cells. Further, the relationship or interdependence of host and pathogen epigenome remains ambiguous during viral reactivation in the physiologically allowed condition of hypoxia. Therefore, we investigated the epigenetic reprogramming of KSHV genome during hypoxic reactivation. We identified a dramatic upregulation in levels of both transcriptions activating as well deactivating methylated histones. Specifically, levels of H3K4Me3, H3K9Me3 and H3K27Me3. Upregulation of these modified histones were restricted to the background of KSHV-positive cells suggesting a coordinated regulation due to hypoxia and KSHV-encoded antigens. KSHV-encoded RTA, vCyclin and vGPCR mediated upregulation of these modified histones. In addition, chromatin sequencing analyses for replication associated proteins such as DNAPol1α and DNA modifying proteins such as DNMTs showed qualitative enrichment on the KSHV genome. These studies increase our understanding of the strategies for epigenetic reprogramming during hypoxic reactivation of the KSHV genome.

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KSHV 2021 Virtual Meeting on
Kaposi's Sarcoma Herpesvirus
(KSHV)
& Related Agents

Poster Session II

Day 3, June 23rd

7:00am-9:00am

P2.1

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P2.2

CRISPR-PITA: A CRISPR/dCas9 Based Assay to Determine Recruitment Relations Between Proteins

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For many nuclear processes directional recruitment of protein complexes is critical for proper function. Here we present CRISPR-PITA (**P**rotein **I**nteraction and **T**elomere Recruitment **A**ssay), an assay that determines the ability of a given protein to recruit any other nuclear factor in protein-protein interaction studies. The protein of interest is directed via CRISPR/dCas9, a dead Cas9 that does not cut DNA, to a repeat sequence, such as telomeres, to obtain dots that are easily detectable by microscopy. The recruitment of nuclear endogenous or viral proteins to these dots can then be visualized using specific antibodies. The latency-associated nuclear antigen (LANA) encoded by Kaposi's sarcoma associated herpesvirus (KSHV, HHV-8) is localized to the viral episomal genomes in infected cells, known as LANA dots, but without the viral genomes LANA is equally distributed in the nucleus. Using the CRISPR-PITA we generated LANA dots in un-infected cells (LANA-telomere-dots). We determined recruitment abilities in CRIPSR-PITA to LANA, methyl-CpG binding protein MeCP2, histone deacetylase 1 (HDAC1), and heterochromatin protein 1 (HP1a). LANA was able to recruit its known interactors ORC2 and SIN3A to LANA-telomere dots. LANA was unable to recruit its known interactor MeCP2 whereas MeCP2 did recruit LANA. Similarly, HDAC1 that interacts with MeCP2 through the methyl-CpG binding domain (MBD) and transcriptional-repression domain (TRD) same as LANA, was unable to recruit MeCP2, but MeCP2 recruited HDAC1. In contrast, HP1a that interacts with the N-terminal domain of MeCP2, was able to recruit MeCP2. We propose that available interacting domains can force this unidirectional recruitment. The TRD of MeCP2 is not available in its DNA-free state, but only available for interaction when MeCP2 dimerizes and tightly bound to methylated DNA. This way, only unidirectional recruitment of LANA by MeCP2 will be permitted. In summary, we describe a broadly applicable protein recruitment assay based on CRISPR/dCas9.

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P2.3

Phosphatase PP2A promotes dephosphorylation of RTA to inhibit KSHV lytic reactivation

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Kaposi's Sarcoma-associated herpesvirus (KSHV) is a human gamma herpesvirus which establishes two different phases in its life cycle, the latency and lytic reactivation. KSHV-encoded replication and transcription activator (RTA), an immediate-early master switch protein, plays a central role in switching the viral latency to lytic reactivation. Overexpression RTA in KSHV infected cells is sufficient to initiate viral lytic reactivation and finally produce viral progeny. Extensive studies have described the mechanisms that RTA functions as a viral transcription factor to activate its downstream viral genes and drive viral lytic reactivation. Some studies have also shown that the phosphorylation of RTA is critical for its function, but the regulatory mechanisms of its phosphorylation and dephosphorylation have not been fully elucidated. In this study, we demonstrated that RTA interacts with the scaffold protein PPP2R1A of phosphatase PP2A. We also showed that both overexpression of PPP2R1A and a pharmacological agonist of PP2A, forskolin, can inhibit expression of lytic genes and impair the production of viral progenies. Moreover, we showed that PP2A can dephosphorylate RTA, which inhibits the functions of RTA in lytic reactivation. Finally, we found that RTA can promote the degradation of PPP2R1A through a proteasome pathway during lytic reactivation process. Taken together, we found the scaffold protein PPP2R1A of phosphatase PP2A is a new binding partner of RTA and PP2A can inhibit viral lytic reactivation through dephosphorylation of RTA, providing new insights into the development of novel antiviral strategies.

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P2.4

Decreased Virion Production of Kaposi's Sarcoma-associated Herpesvirus (KSHV) in HMGB1 Knockout Host Cell through the Compromised Viral Gene Expressions

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Multiple host proteins affect the gene expression of Kaposi's sarcoma associated herpesvirus (KSHV) during latent and lytic replication. Particularly, the high mobility group box 1 (HMGB1) is a highly conserved chromosomal protein inside the cell and a prototypical damage-associated molecular pattern molecule outside the cell. HMGB1 has been implicated to have a pathogenic role in viral infectious diseases and regulate the lytic replication of KSHV. However, its functional effects on the KSHV life cycle in KSHV-infected cells have not been fully elucidated. Here, we explored the role of the intracellular and extracellular HMGB1 in KSHV virion production. We performed a CRISPR/Cas9-mediated HMGB1 knockout in the KSHV-producing iSLK BAC16 cell line. Intracellular HMGB1 formed complexes with various proteins, and the level of HMGB1-interacting proteins changed during latent and lytic replication. We also demonstrated that extracellular HMGB1 enhances lytic replication by phosphorylating JNK. Of note, the expression levels of viral genes were attenuated during lytic replication in iSLK BAC16 cells with HMGB1 knockout, and the production of infectious virions in these cells was significantly lower than that in wild-type cells. Collectively, our results demonstrated that HMGB1 is an important cellular cofactor that affects the generation of infectious KSHV progeny during lytic replication.

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P2.5

Transcriptome-wide siRNA screening identifies long noncoding RNA as novel epigenetic regulator for oncogenic demethylase KDM4A

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Lytic reactivation of Kaposi's sarcoma-associated herpesvirus (KSHV) not only plays an essential role for viral transmission, but it is also a prerequisite of viral tumorigenesis. Regulation of KSHV life cycle is a complex process which can be modulated by the viral proteins and host factors. Despite the importance of epigenetic factor which has been uncovered during the past years, there is still a lot of information about regulation of viral reactivation is unknown at present. Epigenetic regulation of chromatin structure and transcription connects genotype to phenotype. Long non-coding RNA (lncRNA) is emerging as a novel type of epigenetic regulator exhibiting diverse biological functions. In this study, we performed an siRNA screen targeting 82 host lincRNA during KSHV reactivation and analyzed which one reduced viral reactivation. As a result, we identified a long intergenic noncoding RNA (lincRNA) KSHV-induced KDM4A-associated transcript (KIKAT)/LINC01061 which is not only important for KSHV reactivation but also interacts with epigenetic factor KDM4A. We showed that KIKAT/LINC01061 is able to interact with and relocate KDM4A which is able to create an open chromatin environment on promoter region that facilitates transcription factor recognition.

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P2.6

Comparative Efficacy Of Routine Histology, IHC, And PCR Diagnostics for Kaposi's Sarcoma In Sub-Saharan Africa

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OBJECTIVE: Kaposi sarcoma (KS) remains a common HIV-associated cancer worldwide. Despite characteristic skin lesions, KS diagnosis is challenging and hematoxylin-eosin (H&E) alone is not a definitive technique for diagnosis. The current gold standard for KS diagnosis is immunohistochemistry (IHC) staining for latency-associated nuclear antigen (LANA-1) of KSHV. While IHC is increasingly available in Africa, the technique is challenging in terms of expertise, instrumentation, and time. Molecular diagnostic technologies have several potential advantages over IHC, and PCR approaches may serve to complement H&E. We hypothesize that both IHC and PCR will augment KS diagnosis compared to H&E alone. **METHODS**: Our combined team from the Uganda Cancer Institute and Ocean Road Cancer Institute obtained biopsies from clinically suspicious incident KS cases. The biopsies were subjected to three-fold analyses: i) H&E staining for tissue histopathology; ii) IHC for KSHV LANA-1; and iii) real-time or conventional PCR to detect KSHV genomes. We then blindly exchanged samples between institutions and repeated the procedures to evaluate the concordance between sites for the procedures. **RESULTS**: Over 50 suspected KS biopsy samples have been collected. Based on analyses performed to date, concordance between detection of KSHV between IHC and PCR is high, at over 83%. Further, testing with IHC and PCR changed or resolved indeterminant testing by H&E alone in approximately 25% of cases. Evaluation of inter-site concordance of findings based on blind sample exchange is ongoing. **CONCLUSIONS:** Our work to date demonstrates that it is feasible to implement methodologies for IHC and PCR-based detection of KSHV in our setting. IHC and PCR appear to assist resolution of uncertain KS diagnoses based on H&E alone. We also found that collaborative research builds capacity at each site. Ultimately, we hope these molecular techniques will prove to be definitive, cost-effective, and time conservative methods for improving KS diagnosis in SSA.

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P2.7

Characterization of the role of murine 2'-5'-oligoadenylate synthetase-like protein 2 (mOASL2) in murine gammaherpesvirus-68 (MHV68) infection

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Murine gammaherpesvirus-68 (MHV68) is a member of the *Gammaherpesvirinae* subfamily and closely related to Kaposi's sarcoma-associated herpesvirus (KSHV). Due to KSHV's specificity to its human host, MHV68 is a widely used model to study gammaherpesvirus pathogenesis *in vivo*. Both viruses encode the ORF20 protein, a member of the herpesviral UL24 protein family. ORF20 is known to induce cell cycle arrest in the G2-phase [1]. In our previous work, we identified human 2'-5'-oligoadenylate synthetase-like protein (hOASL) as an interacting partner of KSHV ORF20 [2]. hOASL belongs to the OAS protein family and is an interferon-stimulated gene (ISG). Unlike its orthologues hOAS1/2/3, hOASL contains an inactive nucleotidyl transferase (NTase) domain with an inactive CCY motif [3]. It still exerts antiviral activity against some viruses, but its role for gammaherpesviral infection is poorly characterized. There are two murine homologues of hOASL, mOASL1 and mOASL2, whose functions remain to be elucidated. We investigated the role of mOASL2 during MHV68 infection and screened for herpesviral UL24 protein family members that interact with mOASL2. mOASL2 was recently shown to inhibit the DNA sensor cyclic GMP-AMP synthase (cGAS) [3] and we could confirm that type I interferon (IFN) levels were higher in mOASL2 KO cells upon cGAS stimulation. Despite of this elevated type I IFN response in the absence of mOASL2, MHV68 growth was significantly enhanced in mOASL2 KO immortalized bone marrow derived macrophages (iBMDM) compared to wildtype (WT) iBMDM, while growth of murine cytomegalovirus (MCMV) was significantly impaired. Our data suggest that mOASL2 exhibits an antiviral effect during MHV68 infection, which is independent of its inhibitory role on cGAS-mediated type I IFN induction.

(1) The role of ORF20 will be investigated with an MHV68 ORF20stop mutant in WT and mOASL2 KO cells. We are currently investigating further mechanisms of action of mOASL2 during MHV68 infection. Nascimento et al. *Arch Viro*l. 10.1007/s00705-009-0420-y
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P2.8

Recruitment of phospholipase Cγ1 to the non-structural membrane protein pK15 of Kaposi Sarcoma-associated herpesvirus promotes its Src-dependent phosphorylation

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KSHV is associated with Kaposi Sarcoma (KS), Primary Effusion Lymphoma (PEL), the plasma cell variant of multicentric Castleman's Disease (MCD), and an inflammatory cytokine syndrome (KICS). Its non-structural membrane protein, pK15, is expressed in KSHV-infected KS tumor cells and recruits cellular phospholipase C gamma 1 (PLCγ1) to activate downstream signaling cascades such as the MEK/ERK, NFkB and PI3K pathway, thereby contributing to the increased proliferation, migration and the characteristic spindle cell morphology of KSHV-infected endothelial cells. We investigated the recruitment of PLCγ1 to pK15 at the molecular and structural level. We found that a phosphorylated Y^{481}EEVL motif in pK15 preferentially binds into the PLCγ1 C-terminal SH2 domain (cSH2), which is involved in conformational changes occurring during the activation of PLCγ1 by receptor tyrosine kinases. We obtained a crystal structure of the PLCγ1 tandem SH2 (tSH2) domain in complex with a pK15 12mer peptide containing the phosphorylated Y^{481}EEVL motif. This structure showed that the pK15 peptide binds to the PLCγ1 cSH2 domain in a position that is normally occupied by the linker region connecting the PLCγ1 cSH2 and SH3 domains. We also demonstrate that longer pK15 peptides containing the phosphorylated Y^{481}EEVL motif can increase the Src-mediated phosphorylation of the PLCγ1 tSH2 region *in vitro*. This pK15-induced increase in Src-mediated phosphorylation of PLCγ1 can be inhibited with a small pK15-derived peptide which occupies the PLCγ1 cSH2 domain. Our observations suggest that pK15 may act as a scaffold protein to promote PLCγ1 activation in a manner similar to the cellular scaffold protein SLP-76, which has been shown to promote PLCγ1 activation in the context of T-cell receptor signaling.

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P2.9

Iatrogenic Kaposi's sarcoma after immunosuppressive therapy: a retrospective study

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Kaposi's disease (KD) is a multifocal disease. It can occur in an endemic setting: it may be associated with human immunodeficiency virus, or it may occur as a complication of immunosuppression, particularly of iatrogenic origin in transplant patients. This report aims to describe the epidemiological, clinical and therapeutic profile of iatrogenic KD in Morocco in a setting not involving organ transplantation. **Methods**: A retrospective analysis of 84 patients with KD covering 30 years period within this study 23 patients were presenting histologically confirmed iatrogenic KD. **Results:** fifteen men and eight women were included with a mean age of 61 years. All patients received corticosteroids, in combination with cyclophosphamide in three cases, azathioprine in one case and methotrexate in three cases. Mean time of onset of lesions after starting immunosuppressive therapy was 31 months. All cases presented cutaneous lesions and the most common location was the lower limbs. Impaired mucosal membrane was seen in 55.2% of patients and visceral involvement was seen in 11 patients (four patients: lymph nodes, two patients: lung, five patients: gastrointestinal tract). HIV serology tests were negative in all patients but HHV8 serology tests were positive in 78.5% of patients. Treatment consisted primarily of reduction or withdrawal of the immunosuppressant while 3 patients received bleomycine. The outcome was favorable in the majority of cases except one death from hypovolemic shock. **Conclusion**. This report emphasizes the value of regular follow-up and routine dermatological examination of patients on immunosuppressant therapy and suggesting the value of screening for HHV8 infection before initiating such therapy.

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P2.10

Multiplex immunohisochemistry reveals PROX1 and SOX18 co-expression with KSHV latent and lytic markers in KS biopsies

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BACKGROUND. Kaposi's sarcoma tumor mass is composed by spindle cells, thought to originate from KS associate herpesvirus (KSHV)-infected lymphatic endothelial cells (LEC). Interestingly, LEC support a unique viral program with spontaneous expression of latent and lytic markers, high KSHV genome copies and release of infectious virus (left schematics). We have studied the LEC-specific factors responsible for this viral expression program and identified SOX18 and PROX1 to be instrumental for KSHV high genome copies and spontaneous lytic gene expression, respectively. Mechanistically, both transcription factors are recruited to the viral genome and while SOX18 participates to KSHV genome replication, PROX1 enhances the ability of ORF50 to trigger the lytic cycle (right panels in the schematics; Gramolelli et al, Cancer Research 2020). **AIM OF THE STUDY.** To assess by immunohistochemistry on KS biopsies SOX18 and PROX1 expressions in correlation with markers of latent (LANA) and lytic (K8.1) KSHV infection. **CONCLUSIONS.** Both single staining and multiplex immunohistochemistry on KS biopsies showed that SOX18 and PROX1 are expressed in the majority of the spindle cells and their distribution correlates with LANA and K8.1, markers of latent and lytic infection, respectively. This suggests that also in vivo SOX18 and PROX1 are important to support the tumorigenic KSHV life cycle in spindle cells and may represent suitable and novel therapeutic targets.

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P2.11

The cellular DNA damage response proteins SMARCAL1 and RPA are recruited by KSHV LANA and impact viral lytic replication

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Kaposi's sarcoma associated herpesvirus (KSHV) establishes a lifelong persistent latent infection in affected individuals. The most abundant latent viral protein, latency associated nuclear antigen (LANA), plays an important role in latent replication by tethering the viral genome to the host chromatin during mitosis. LANA interacts with many host proteins to mediate its role in the viral life cycle. We and others have identified the cellular proteins RPA and SMARCAL1, which are involved in DNA replication and damage response, as LANA interactors [1, 2]. RPA is recruited upon DNA damage or replication stress and in turn recruits SMARCAL1 to stalled replications forks. Using pulldown and co-immunoprecipitation assays we found that SMARCAL1 is indirectly interacting with LANA via RPA2 and that the RPA2/SMARCAL1 complex interacts with the ten N-terminal amino acids of LANA that also mediate binding to H2A/B. We also demonstrate via Immunofluorescence that this interaction is dynamic and changes during the viral life cycle. By silencing (siRNA) and/or deleting (CRISPR/Cas9) RPA2 and SMARCAL1 from infected cells, we show opposing roles of these proteins for productive KSHV replication. RPA2 is required for efficient replication and SMARCAL1 restricts it. Moreover, via immunoblot we demonstrate that SMARCAL1 protein levels are decreasing upon KSHV reactivation, probably at least partly as the result of proteasomal degradation. These observations are reminiscent of previously reported roles of RPA in EBV, HSV-1, HCMV, and AAV infection and also of the recently demonstrated degradation of SMARCAL1 during Adenovirus replication [3]. This may therefore suggest that the involvement of RPA and SMARCAL1 in virus replication is conserved across different families of DNA viruses.

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3. Nazeer et al. 2019, J. Virol.

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P2.12

Epidemiological Profile and Clinical Features of Kaposi Sarcoma in Rabat (Morocco)

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Kaposi's sarcoma (KS) is a public health problem in most of sub-Saharian African countries. In Morocco due to the insufficient data regarding this disease, it seemed important to us to collect all the Moroccan published studies and compare them to our study in order to have an overview of the epidemiological situation and clinical features of KS in our country. We performed a retrospective analysis of 85 Kaposi patients seen between January 2002 and April 2021 in the Dermatology Department of Ibn Sina Hospital (Rabat, Morocco). All types of Kaposi sarcoma (classic, iatrogenic and AIDS-related KS) confirmed by histology were examined and the epidemiological and clinical features were assessed. The average age of our patients was 61.5±10 years old, the male-to-female ratio 2.3. The mean duration of disease was 35.4 months, the most common clinical subtype was: classic KS 71.7%, iatrogenic KS 25.8%, two cases AIDS-related KS. The main clinical aspect of the lesions was violaceous hyperkeratotic papulonodules in all of our patients, vegetative tumors in 5 patients, 3 cases of lower limb ulcer. Lower limbs were the main location 70%, the mucosal involvement was found in 58.8% (24oral, 2nasal 18genital, 6ocular), onychodystrophy was found in 23% of patients. Lymphoedema was present in 65% cases. Visceral disease was observed in 30% of cases in the lymph nodes, gastrointestinal tract, spleen and lungs. Treatment options included: bleomycine (50 patients), Electrochemotherapy 5, surgery 4, cryotherapy 2, vinblastine 5 cases, radiotherapy 1, abstention in 3 patients, chemotherapy 3 patients. Our data join the results of others Moroccan studies. KS remains an unknown disease by many patients but also some doctors, which explains the delay in diagnosis and the development of visceral forms, moreover the therapeutic options being limited, and the use of long-term corticosteroid therapy bought over the counter worsen the situation in our Country.

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P2.13

KSHV Uses Viral IL-6 to Exploit Monocyte Inflammatory Response

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), multicentric Castleman disease, and KSHV inflammatory cytokine syndrome (KICS), a severe inflammatory disease associated with increased IL-6, IL-10, and a KSHV-encoded IL-6 homolog (vIL-6). Unlike human IL-6, vIL-6 can directly bind and activate gp130 and the STAT-NF-kB pathway from inside the cell without other receptor subunits. Given the wide distribution of gp130, vIL-6 is thought to play an important role in inflammatory symptoms characteristic of KSHV-mediated diseases. Here, we studied vIL-6 biological function by generating a vIL-6 Stop KSHV infection model. Single-cell RNA sequencing analysis of de novo KSHV infection of human peripheral blood mononuclear cells showed that CD14+ monocytes were preferentially infected and supported KSHV lytic replication with vIL-6 expression. Importantly, KSHV infection promoted monocyte survival in a manner dependent on functional vIL-6 expression. Moreover, after prolonged vIL-6 stimulation, the monocytic THP-1 cell line acquired a highly inflammatory phenotype in association with constitutive up-regulation of oncogenic transcription factors, STAT3 and BATF, known to play a role in transcription reprogramming in PEL. Also, target gene identification of vIL-6 with thiol (SH)-linked alkylation for the metabolic sequencing (SLAM seq) demonstrated a remarkably similar profile with human IL-6 stimulation. These results suggest that vIL-6 plays an important role in monocyte survival and differentiation during KSHV infection. We speculate that prolonged monocyte lifespan would increase the magnitude of inflammatory response and the likelihood of infected monocytes to differentiate into long-lived macrophages. This mechanism may contribute to the development of recurrent chronic inflammation seen in KS patients.

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P2.14

Image-Based Analysis of Latent KSHV DNA Replication Using Engineered KSHV

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In the latent phase of infection, herpesvirus exist and replicate as discrete episomes in the host cell nucleus along with cell division. While we understand that latency-associated nuclear antigen (or LANA) is a viral protein that is important for maintaining Kaposi sarcoma-associated herpesvirus in the latent state by physically tethering KSHV episomes to host chromosomes. The process and mechanisms that determine the number and distribution of viral episomes throughout the host cell cycle requires to observe individual episomes in live cells at single cell resolution. We introduce a novel tool and approach to studying this system by engineering KSHV with a variety of fluorescent proteins, including mCherry-tagged LANA. Using high performance 3D live cell microscopy, we can readily identify these reporter KSHVs as fluorescent LANA "dots" in the nuclei of individual host cells – and collect statistical data on their number and distribution during various cell states. Here we utilize the reporter KSHV to examine KSHV episome replication along with cell division. We monitored KSHV episome numbers by taking live cell images with 3D stacks for 3 days. The results suggested that distribution of viral episomes (LANA dots) to daughter cells significantly varied at every cell division, and they are not equally distributed. Increasing hyglomycin, which is used for selection of BAC transfected cells, gradually increased episome copy numbers per cells. On the other hand, removing hygromycin rapidly reduces KSHV episomes only to the basal levels, suggesting presence of preferential tethering sites where KSHV episome could be maintained more stably. Incubation of acyclovir did not affect KSHV episome copy numbers. We are currently analyzing effects on KSHV latent DNA replication by DNA damaging agents and inhibitors. We are also analyzing the LANA tethering sites and redistribution of daughter cells by examining distance of each of LANA dots.

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P2.15

Development of a concomitant surface, intracellular and RNA flow cytometry assay for detecting and phenotyping KSHV-infected cells

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Combining multiparameter flow cytometry with fluorescence *in situ* hybridization to detect RNA transcripts (PrimeFlow™ RNA assay), we investigate the expression of gammaherpesvirus RNA and protein molecules on a single-cell level. For this assay, we combine surface and intracellular flow cytometry with fluorescence *in situ* hybridization to detect KSHV protein and RNA transcripts, as well as host-cell subset and activation markers, during latent and lytic infection. Probe sets recognizing RNA sequences of four KSHV genes were developed: three mRNAs [ORF73 (LANA, latent), K12 (latent) and K8.1 (lytic)] and one ncRNA (polyadenylated nuclear, PAN). Binding of the KSHV probe sets and antibodies were assessed in three lymphoma cell lines isolated from body cavities [Ramos (KSHV-), Trex-BCBL-1-Rta and VG-1 (KSHV+)], during latency and after reactivation with doxycycline (Dox) or sodium butyrate. Specificity and sensitivity were determined for each probe set and antibody reagent. Viral probe set multiplexing and host cell subset and activation marker incorporation were also assessed. In both KSHV+ cell lines, high levels of viral LANA protein and ORF73, K12 and PAN RNA were detected, despite varying KSHV subtypes. Following a 24-hour exposure to Dox, both the K8.1 lytic transcript and protein greatly increased in KSHV+ cell lines. This modified PrimeFlow™ RNA assay can specifically and sensitively detect both KSHV RNA transcripts and protein in lymphoma cell lines, with little to no background. Additionally, detection of viral transcripts (especially PAN) proved to be more sensitive for identifying KSHV infection, than viral protein. These results suggest this assay could be useful for identifying KSHV-infected cells in patient samples, as well as evaluating viral and host-cell RNA and protein dynamics, to better understand KSHV pathogenesis.

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P2.16

The role of histamine-related signaling in promoting viral lytic reactivation and oncogenesis

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Kaposi's sarcoma-associated herpesvirus (KSHV) causes several human cancers, such as Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL). Current treatment options for KSHV infection and virus associated diseases are sometimes ineffective, therefore, more effectively antiviral agents are urgently needed. As a herpesvirus, lytic replication is critical for KSHV pathogenesis and oncogenesis. After performing a high-throughput screening of small-molecule compound library that consisted of 1280 Food and Drug Administration (FDA)-approved drugs, we have identified hit compounds that effectively inhibited KSHV virion production. Interestingly, 3 of these drugs target histamine receptors or signaling. Our data further confirmed that antagonists targeting different histamine receptors (HxRs) displayed excellent inhibitory effects on KSHV lytic replication from induced iSLK.219 or BCBL-1 cells. In contrast, histamine and specific agonists of HxRs promoted viral lytic replication from induced iSLK.219 or KSHV-infected primary cells. Mechanistic studies indicated that downstream MAPK and PI3K/Akt signaling pathways were required for histamine/receptors mediated promotion of KSHV lytic replication. Direct knockdown of HxRs in iSLK.219 cells effectively blocked viral lytic gene expression during induction. Using samples from a cohort of HIV+ patients, we found that the KSHV+ group has much higher levels of histamine in their plasma and saliva than the KSHV- group. Additionally, we also examined the expression of HxRs in AIDS-KS tissues. Taken together, our data have provided novel insights into the molecular bases of histamine-related signaling that contribute to reactivation of this oncogenic herpesvirus and its pathogenesis.

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P2.17

CRISPR Interference Efficiently Silences Latent and Lytic Viral Genes in Kaposi's Sarcoma-Associated Herpesvirus-Infected Cell

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Uncovering viral gene functions requires the modulation of gene expression through overexpression or loss-of-function. CRISPR interference (CRISPRi), a modification of the CRISPRCas9 gene editing technology, allows specific and efficient transcriptional silencing without genetic ablation. CRISPRi has been used to silence eukaryotic and prokaryotic genes at the single-gene and genome-wide levels. Here, we report the use of CRISPRi to silence latent and lytic viral genes, with an efficiency of ~80–90%, in epithelial and B-cells carrying multiple copies of the Kaposi's sarcoma-associated herpesvirus (KSHV) genome. Our results validate CRISPRi for the analysis of KSHV viral elements, providing a functional genomics tool for studying virus–host interactions.

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P2.18

Influence of Cytokine Signaling on KSHV Transmission

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Saliva is widely accepted as the primary route of person-to person transmission of KSHV. However, the exact mechanisms for viral transmission and factors influencing susceptibility and the establishment of infection remain unclear. This gap in our understanding negatively affects our ability to identify efficient strategies to decrease KSHV transmission. Viral manipulation of the inflammatory environment of the human host profoundly alters the pathogenesis of KSHV-associated diseases. However, the contribution of cytokines to KSHV transmission and the early stages of infection remain vague. Our laboratory uses a unique ex vivo culture system to study the establishment of KSHV infection in human B lymphocytes derived from human tonsils. In this study, we investigate the role of cytokines in influencing the susceptibility of tonsil lymphocytes to KSHV infection and explore the mechanisms behind these influences. Specifically, we will present data examining the role of interleukin IL-21(IL-21) in KSHV infection of B lymphocytes and show that manipulation of this cytokine influences the establishment of KSHV infection in a lineage-specific manner. We also explore the immunological subsets contributing to IL-21 signaling in the human tonsil and their role in the establishment of KSHV infection in plasma cells and other B cell lineages. This data provides novel and critical insight into KSHV transmission by establishing the role of IL-21 in the early stages of KSHV infection in the human immune system.

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P2.19

The Indian perspective of KSHV associated malignancy with a view of a potential natural therapeutic application

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AIDS associated malignancies are major cause of co-morbidity among AIDS patients worldwide. KSHV is an important agent associated with such cases. India is the third largest HIV epidemic region in world. But HIV-AIDS associated KSHV infection is underrated topic here. Our investigation from reviewing all case reports shows that, from 1993 to 2019, about 25 cases of Kaposi's sarcoma have been reported from different regions in India, all of which were seropositive for HHV-1. On the other hand, tuberculosis (TB) is the most common opportunistic infection in India which has been reported as a co-existing factor with almost all the HIV patients. Interestingly we found that KS patients from India have been reported with reactivation of previous history of tuberculosis or developed during HIV infection. Prolonged application of ART in such patients may results in the development of a clinical complication known as immune reconstitution inflammatory syndrome (IRIS). Imbalanced cellular stress response as chemotherapeutic after effect, might be the causes of such co-infected (HI V-TB-KS) malignant cells. Reactive oxygen species (ROS) has been found as the key determining factor for latent-lytic balance in KSHV infected cells whose balance play critical role in oncogenesis. Due to these scenarios, there are no such potential anti-cancerous drugs clinically effective enough against KSHV associated malignancies. Thus, to combat such KSHV infected malignancies, we have to strategically design a traditional therapeutic approach which can effectively attack the "cancer cell stress management system" with minimal after effect of stress response. We clearly show that two very common naturally derived antioxidants (ROS scavengers) can act both as pro-oxidant or antioxidant in order to manage the imbalanced stress management system during KSHV oncogenesis by utilizing the autophagy and drives apoptotic cell death of HIV associated/non-associated KSHV infected cell lines. Our approach reveals a cost-effective stress balancing therapeutic strategy in natural way by which the ART related drawbacks in HIV and TB associated patients from poor socioeconomic background can be avoided in long term.

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P2.20

vIRF-1 interacts with the autophagy-competent mitochondrial translational elongation factor TUFM to inhibit caspase-8-mediated apoptosis

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We previously demonstrated that vIRF-1 plays an important role in the inhibition of mitochondria-mediated apoptosis induced by HHV-8 lytic replication via multiple mechanisms, that is, the direct inhibition of proapoptotic proteins or the promotion of NIX-mediated mitophagy. In an attempt to understand the role of vIRF-1 on the mitochondrial detergent-resistant microdomain (mDRM), which is a venue for mitochondria targeting of vIRF-1, we identified the mitochondrial translation elongation factor Tu, encoded by the *TUFM* gene, as a novel vIRF-1 binding protein using far-western blotting and mass spectrometry. TUFM depletion potentiated caspase-8-mediated apoptosis induced by vIRF-1 depletion in lytic BCBL-1 cells along with significant accumulation of altered mitochondria. In addition, TUFM depletion promoted caspase-8 activation induced by treatment with TNF-related apoptosis-inducing ligand (TRAIL) in cancer cells, potentially via dysregulation of mitochondrial dynamics and mitophagy, suggesting that TUFM is involved in mitophagy activation to inhibit mitochondria-mediated apoptosis. Importantly, we found that dimerization of TUFM via the N-terminal GxxxG motif is required for interaction with the autophagy core machinery ATG12-ATG5 conjugate and inhibition of TRAIL-induced caspase-8 activation. Furthermore, we discovered that the autophagy-competent (dimerized) form of TUFM was subject to ubiquitin-proteasome-mediated degradation but stabilized upon vIRF-1, mitophagy or autophagy activation in mDRM. Taken together, these results suggest that vIRF-1 may play a role in the promotion of TUFM-mediated mitophagy in mDRM for successful virus lytic replication.

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P2.21

GRWD1-WDR5-MLL2 Epigenetic Complex Mediates H3K4me3 Marks and Is Essential for KSHV Induced Cellular Transformation

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Infection by Kaposi's sarcoma-associated herpesvirus (KSHV) is causally associated to the development of several cancers. Despite intensive studies, the mechanism of KSHV-induced oncogenesis remains unclear. By performing a genome-wide CRISPR-Cas9 screening, we have identified a set of cellular genes related to epigenetic regulation that are essential for KSHV-induced cellular transformation. Several of them have not been implicated in cancer before. Examination of TCGA datasets revealed that the expression of these epigenetic factors predicts patient survival in numerous types of cancer, indicating their essential roles in cancer development. Using a KSHV-induced cellular transformation model of primary mesenchymal stem cells, we revealed the global epigenetic remodeling of KSHV-transformed cells (KMM) compared to the matched primary cells (MM). Knockdown of one of the identified genes Glutamate Rich WD Repeat Containing 1 (GRWD1), a WD40 family protein upregulated in KMM cells, not only inhibited cell proliferation, cellular transformation and tumor formation but also caused down-regulation of global H3K4me3 marks of KMM cells. Protein pull-down identified WDR5, the core protein of H3K4 methyltransferase complex and several H3K4me3 methyltransferases including MLL2 as the GRWD1-interacting proteins. Co-immunoprecipitation and confocal microscopy analyses confirmed that GRWD1, WDR5 and MLL2 interacted with one another in the same protein complex. Importantly, knockdown of WDR5 and MLL2 phenocopied GRWD1, and altered the expression of a similar set of genes and caused globally reduction of H3K4me3 marks. RNA-seq and ChIP-seq analysis further identified common and distinct cellular genes and pathways that were regulated by GRWD1, WDR5 and MLL2. These results indicate that KSHV upregulates GRWD1 to hijack the GRWD1-WDR5-MLL2 epigenetic complex and increases H3K4 tri-methylation, which is essential for KSHV-induced cellular transformation and tumorigenesis. Our work has identified an epigenetic complex as a novel therapeutic target for KSHV-induced cancers.

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P2.22

Dynamic changes in antibody repertoire of MHV68-infected B cells

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Murine gammaherpesviruses 68 (MHV68) is a rodent pathogen frequently used as an animal model to study the pathogenesis of human gammaherpesviruses (γHV) Kaposi's sarcoma herpesvirus (KSHV) and Epstein-Barr virus (EBV). During acute infection, like other HV, MHV68 targets and rapidly replicates in respiratory tract epithelial cells. Subsequently, the virus infects B lymphocytes and latently expands in germinal center (GC) B cells. Normally the GC compartment undergo antibody diversification through somatic hypermutation (SHM) and class switch recombination (CSR) in conjunction with an antigen driven selection process. This process gives rise to the long-lived memory B cells where MHV68 resides in long term. It is unclear how γHV impacts the selection and diversification process of infected GC cells. To assess the impact of gammaherpesvirus infection on these processes we utilized an MHV68 mouse infection model. We analyzed the immunoglobulin repertoire of infected cells in the spleen and mediastinal lymph nodes of individual mice. Using single cell immunoglobulin (Ig) sequencing we determined the repertoire of infected cells in different organs at various timepoints post infection. We find that infected B cells express Igs that are engaged in ongoing diversification and antigen selection. Interestingly, MHV68 resides in B cells with distinct repertoire with biased utilization of IGHV genes and lambda light chain. By analyzing repertoire over various time points post infection we find differences in the repertoire expressed by infected cells particularly at late time points. This data suggests the virus actively subverts the GC diversification and selection process to exist in B cells with distinct repertoire over time.

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P2.23

The Portal Vertex of KSHV Promotes Docking of Capsids at the Nuclear Pores

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The KSHV capsid, like other herpesviral capsids, is icosahedral and includes 11 penton vertices and a single portal vertex composed of 12 protein subunits encoded by open reading frame (ORF) 43. The portal enables packaging of the viral genome into the preformed ball-like procapsid, and release of the genome into the nucleus through the nuclear pore complex (NPC). Capsid vertex-specific component (CVSC) tegument proteins are organized on the capsid penton vertices and are enriched on the portal vertex. These proteins mediate docking of the capsid at the nuclear pore. Whether and how the portal vertex is preferably selected for docking at the NPC prior to DNA release is unknown. Furthermore, it is not understood if and how the portal affects the structure and occupancy of the CVSC on the capsid vertices. Here, we investigated the docking of incoming ORF43-null KSHV capsids at the NPCs and describe a significantly lower fraction of capsids attached to the nuclear envelope compared to wild-type (WT) capsids. Yet, the mutated virus did not present a delayed intracellular trafficking kinetics. Like WT capsids, nuclear envelope-associated ORF43-null capsids co-localized with different nucleoporins (Nups) and did not detach upon salt treatment. Inhibition of nuclear export did not alter the docking of WT capsids. Although having a lower fraction of docking at the nuclear membrane, ORF43-null capsids were strongly bound. As ORF43-null capsids exhibited lower extent of association with the NPCs, we conclude that, although not essential the portal promotes the interaction of the CVSC proteins with Nups. Furthermore, these findings suggest that there is a certain degree of probability for WT capsids to dock at the nuclear envelope with an incorrect penton vertex, resulting in an infection 'dead end'.

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P2.24

Protein-RNA interactome analysis reveals wide association of KSHV ORF57 with host non-coding RNAs and polysomes

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Kaposi's sarcoma-associated herpesvirus (KSHV) ORF57 is a viral post-transcriptional regulator during KSHV lytic infection. Using ORF57-CLIP (Cross-linking Immunoprecipitation) we showed ORF57 association with several viral RNAs, including ORF59, K8, PAN, and vIL6. We recently utilized an affinity-purified anti-ORF57 antibody and conducted the ORF57-CLIP in combination with RNA-seq (CLIP-seq) to analyze the genome-wide RNA transcripts associated with ORF57 in reactivated BCBL-1 cells. Mapping of the CLIPed RNA reads to the human genome (hg19) revealed that most of the ORF57-associated RNA reads (83.3%) were mapped to the mature rRNA in the order of 5.8S > 18S > 28S rRNAs. The remaining RNA reads were mapped to several classes of non-coding RNAs and protein-coding mRNAs. We found ORF57 binds several host lncRNAs, including LINC00355, LINC00324, and LINC00839 of which are involved in cellular growth. ORF57 also binds the snoRNAs responsible for rRNA modification. We validated sixty-seven of these snoRNAs-ORF57 interactions in the cells by ORF57-CLIP in combination with Arraystar snoRNA-RT-qPCR arrays. Interestingly, most of rRNA binding sites (BS) identified for ORF57 binding in our CLIP-seq analyses were the same BS for snoRNAs as exemplified by snoRA71B. We confirmed the ORF57-CLIPed snoR71B RNA by Northern blot analysis using an ^{32}P-labeled oligo probe antisense to the snoR71B region interacting with the 18S rRNA. Using the RNA oligos derived from the rRNA regions that ORF57 binds for oligo pulldown-Western blot assays, we selectively verified ORF57 interaction with 5.8S and 18S rRNAs through the BSs identified by ORF57 CLIP-seq. In 4.5%-45% sucrose gradient polysome profile assays, ORF57 was found in co-association with monosomes and polysomes along with PABPC1 and prevented Ago2 from association with polysomes, indicating a functional correlation with ORF57 binding and suppression of RISC activities for ORF57 promotion of gene expression.

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P2.25

African Endemic and Epidemic Kaposi sarcoma in Tanzania: A comparative study in a cohort of 313 patients

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Background: African endemic-Kaposi's sarcoma (EnKS) and HIV-1 associated epidemic-KS (EpKS) are two common cancers in sub–Saharan Africa. This study evaluated differentials in KS disease presentation and health care utilization in SSA. **Methods:** This prospective cohort consists of 313 clinically suspected KS patients in Tanzania recruited between 2015 and 2020. Sociodemographic and behavioral characteristics were documented using a questionnaire. Clinical examination was performed at baseline and during follow-up visits. Chi-square tests were used to test for associations between categorical variables. The Wilcoxon non-parametric test was used to compare between EnKS and EpKS patients. P-values <0.05 were considered significant. **Results:** Out of 313 suspected KS patients, 228 histologically diagnosed KS patients were included in the final analysis. EnKS patients were mainly of male gender, significantly older, and more likely to be involved in the farming activities compared to EpKS patients (P<0.05). The median CD4 count was 790 and 191 for EnKS and EpKS respectively. The major KS morphotype was nodular KS on extremities with mucosal involvement in ~20% for both forms of KS. Discharge and foul-smelling from the KS lesions were recorded at baseline at similar rates (~35%). After 5 years of the study, loss to follow up was at 48% and 110 patients (18 EnKS and 92 EpKS) had at least one follow-up visit. Six months after treatment for KS, ~75% of patients reported reduction in number and size of the lesions. The duration of disease in EnKS was longer compared to EpKS. The average time to start ART was 1 year since diagnosis of HIV for EpKS. **Conclusions:** The disease presentation was largely comparable for the two forms of KS. There was a delay in seeking care for both forms of KS, and this finding highlights the need to explore barriers in access to health care for KS patients.

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P2.26

Pomalidomide and liposomal doxorubicin for Kaposi sarcoma +/- other KSHV diseases

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Kaposi sarcoma herpesvirus (KSHV) is the causative agent of Kaposi sarcoma (KS), a multicentric angioproliferative tumor, a form of multicentric Castleman disease (KSHV-MCD) and KSHV inflammatory cytokine syndrome (KICS). KS can be challenging to treat when it occurs with KSHV-MCD or KICS, resulting in high mortality rates. Liposomal doxorubicin (dox), a chemotherapy, and pomalidomide (pom), an immunomodulatory drug, are FDA-approved therapies for KS. The safety and activity of the combination (pom/dox) in KS alone or with KSHV-associated diseases are unknown. The primary objective of this Phase I/II study was to evaluate safety and tolerability of pom/dox in 2 groups of participants with KS: Group I (G1)- KS alone; Group II (G2)- KS with concurrent active KSHV-MCD or KICS. Patients received dox at 20 mg/m^2 intravenously on day 1 of a 28-day cycle combined with pom once daily on days 1 to 21 at escalating dose levels (DL) (I - 2mg, II - 3mg, or III- 4mg) in a 3+3 design. Participants received 81mg of aspirin daily as thromboprophylaxis. Thirty-four cisgender men, all with severe (T1) KS [21 patients (62%) in G1 and 13 patients (38%) in G2] were treated; 32 (94%) were HIV-infected and 22 (65%) had prior chemotherapy for KS. There were no dose-limiting toxicities (DLTs) at DLIII for G1. In G2, grade 3 rash and pharyngeal edema were DLTs observed at 3mg of pom (DLII). Among evaluable participants receiving $\geq$2 cycles, 17/21 patients in G1 had a KS response (16 partial and 1 complete) (81% [95% confidence interval (CI) 58-95%]) and 5/10 patients in G2 had a KS response (4 partial and 1 complete) (50% [95% CI 19-81%]. In participants with KS alone, pom/dox was well-tolerated and active. Among those with KS and KSHV-MCD or KICS, the combination was active but less well-tolerated.

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P2.27

The cellular Notch1 Protein Promotes KSHV reactivation in a Rta-dependent manner

Jennifer DeCotiis-Mauro, **Sun M. Han**+, Helena Mello+, Corey Goyeneche, Giuseppina Marchesini-Tovar, Lianhua Jin, and David M. Lukac

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The cellular Notch signal transduction pathway is intimately associated with infections by gamma-herpesviruses like Kaposi's sarcoma-associated herpesvirus (KSHV). Reactivation of KSHV from latency requires the viral lytic switch protein, Rta, to form DNA-bound complexes with the cellular protein RBP-Jk, which is the DNA binding component of the Notch pathway. The KSHV genome contains >100 RBP-Jk motifs, yet we show that none of the four isoforms of activated Notch is sufficient to productively reactivate the virus from latency in a highly quantitative trans-complementing reporter virus system. The insufficiency of Notch1 extends to alleles known to have varied inherent transcriptional activity. Constitutive Notch activity is essential for KSHV pathophysiology in models of Kaposi's sarcoma (KS) and Primary Effusion Lymphoma (PEL), and we demonstrate that Notch1 is also constitutively active in infected Vero cells. Despite the inability of activated Notch to reactivate KSHV, broad inhibition of Notch1-4 with gamma secretase inhibitor (GSI) or expression of dominant negative (DN) mastermind-like1 (MAML1) coactivators dramatically debilitate production of infectious KSHV. Notch1-specific siRNAs also partially reduce reactivation and implicate Notch1 as one component of proviral Notch activity. The negative effect of DN-MAML1 suggests that Notch1 functions in a MAML1/RBP-Jk/DNA complex to support reactivation. Ectopic NICD1 also enhances production of mature virus in VPA-induced reactivation. However, our observation that Notch1 knockdown is not as potent as broad Notch inhibition suggests that the other Notch isoforms are also proviral. Importantly, GSI's effect on virus production occurs without affecting cell growth. In infected PEL cells, we show that reduction of vDNA synthesis by GSI is associated with gene specific reduction of viral transcription. We conclude that constitutive Notch activity is required for robust production of infectious KSHV in a promoter-specific, Rta-dependent fashion.

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KSHV 2021 Virtual Meeting on
Kaposi's Sarcoma Herpesvirus
(KSHV)
& Related Agents

Session VI:

Replication and Therapeutics

9:30am-11:00am

6.1

DNA processing by the bifunctional SOX nuclease plays a key role in KSHV genome replication and gene expression

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Although the exact mechanism by which herpesviral DNA replicates remains unclear, branched structures have been observed by electron microscopy that are presumably replication intermediates that must be resolved before unit length genomes are packaged. In KSHV and other herpesviruses such as herpes simplex virus 1 (HSV-1) and Epstein Barr virus (EBV), resolution of these branched structures requires a virally encoded alkaline exonuclease (AE) termed SOX in KSHV. In gammaherpesviruses, SOX is a bifunctional enzyme that cleaves cytoplasmic mRNA to promote host shutoff and processes viral DNA in the nucleus during genome replication. Prior work has established that the DNase activity of SOX and its homologs appears necessary to maintain viral genome integrity during replication, although little is known about the nature of the preferred DNA substrate(s), how the dual DNase and RNase activities are coordinated by residues within and outside of the catalytic core and whether SOX engages in functional DNA binding during viral genome replication. We addressed each of these questions through an integrated structure-function analysis of the SOX protein using *in vitro* measurements with purified components as well as functional assays in SOX expressing cells and in the context of KSHV infection. We identified several non-catalytic residues that contribute to DNA processing as well as residues essential for catalytic activity on both RNA and DNA. Notably, while SOX binds DNA with nanomolar affinity, this binding appears dispensable both for its RNA targeting and, surprisingly, for its role in genome replication. We engineered several of these point mutants into the KSHV genome and found that KSHV mutants lacking functional SOX are defective in replicating the viral genome, viral gene expression and producing infectious virions. Collectively, our findings provide new insight into mechanisms underlying the role of SOX in viral DNA processing and KSHV replication.

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6.2

Glycoprotein M is an Essential Factor of Rhesus Macaque Rhadinovirus Viral Egress

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The development of a vaccine for KSHV would yield substantial benefits globally; yet one major roadblock has been the lack of an animal model where KSHV-specific therapeutics can be stringently tested. Rhesus macaque rhadinovirus (RRV) is a gammaherpesvirus closely related to KSHV and shares many aspects of KSHV infection and pathogenesis—including the ability to cause B cell lymphomas in susceptible rhesus macaques. To adapt the RRV animal model for future KSHV-specific studies, a better understanding of the similarities and differences between these viruses is required. One traditional vaccine strategy involves the development of antibodies capable of blocking KSHV infection by targeting viral glycoproteins, which have critical roles for virus entry. Importantly, virus neutralization has already been achieved in several *in vitro* experiments. Since RRV encodes a set of highly homologous glycoproteins to those of KSHV, then it might be possible to generate KSHV glycoprotein chimeric RRV, which would significantly facilitate the development and preclinical evaluation of KSHV-specific therapeutics *in vivo*. To this end, we have been focusing on one of the least understood glycoproteins in both KSHV and RRV—glycoprotein M (gM). By utilizing BAC recombination, we generated a gM-nonsense recombinant RRV clone. Upon transfection of this recombinant virus genome into rhesus fibroblasts, we discovered that gM was essential for infectious virus production. These results are highly reminiscent of the severe consequences of knocking out gM homologs in other herpesviruses. Furthermore, we have also generated a KSHV gM-chimeric RRV clone and are testing its ability to rescue infectious virus production. To validate these results, we are also developing RRV- and KSHV-specific, gM-complementing cell lines. Collectively, these studies reveal the functional equivalency of these viral glycoproteins and will provide new knowledge about the biological contributions of gM to rhadinoviruses, which may support the development of a vaccine or new antivirals.

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6.3

Genome-wide regulation of KSHV RNA splicing by RNA-binding protein ORF57

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Splicing plays an essential role in KSHV gene expression. We previously showed that KSHV ORF57 is a viral splicing factor to promote viral lytic gene expression. To uncover a genome-wide ORF57 regulation of KSHV RNA splicing, we compared viral RNA splicing profile in BCBL-1 cells carrying a wild-type (WT) KSHV genome with the cells carrying an ORF57 knock-out (KO) genome during viral lytic infection. By RNA-seq analyses, we identified hundreds of novel viral RNA splicing events, including the splicing events spanning large parts of the genome. Interestingly, we found that ORF57 KO affects RNA splicing of numerous viral transcripts either by increasing or decreasing the targeted splicing events. Among them, we have identified ORF70 (thymidylate synthase) and K3 (ubiquitin E3 ligase) expressed as a bicistronic ORF70-K3 RNA bearing two introns, of which intron 1 is positioned in the ORF70 coding region and intron 2 in the intergenic region. ORF57 regulates the alternative intron 1 splicing of the bicistronic ORF70-K3 RNAs. In the WT cells, most ORF70-K3 transcripts normally exhibit intron 1 retention to maintain an intact ORF70 ORF, whereas in the ORF57 KO cells, loss of viral ORF57 expression led to a substantial increase of the intron 1 splicing, but, had no effect on the constitutive intron 2 splicing. Using a minigene containing the ORF70-K3 locus in expression and RNA splicing assay in HEK293T cells in the presence or absence of ORF57, we further confirmed the observed ORF57 regulation of ORF70-K3 bicistronic RNA splicing independently of other viral factors. Viral ORF57-mediated intron 1 retention in the ORF70 ORF promoted the production of ORF70 protein, but significantly reduced the protein expression of K3. Altogether, we conclude that ORF57 regulates alternative splicing of ORF70-K3 bicistronic RNA to control K3-mediated immune evasion and ORF70 participation of viral DNA replication in viral lytic infection.

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6.4

The Expression and Nuclear Retention Element (ENE) of Polyadenylated Nuclear (PAN) RNA is not required for productive lytic replication of KSHV

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KSHV is an oncogenic human gammaherpesvirus and the causative agent of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL) and multi-centric Castleman's disease (MCD). During reactivation, viral genes are expressed in a temporal manner. These lytic genes encode for transactivators, core replication proteins, or structural proteins. During reactivation, other viral factors are expressed that are required for lytic replication. The most abundant viral transcript is the long non-coding RNA (lncRNA) known as polyadenylated nuclear (PAN) RNA. lncRNAs have diverse functions, including regulation of gene expression and immune response. PAN possesses two main cis-acting elements, the Mta response element (MRE) and the expression and nuclear retention element (ENE). While PAN has been demonstrated to be required for efficient viral replication, the function of these elements within PAN still remains unclear. Our goal was to determine if the ENE of PAN is required in the context of infection. A KSHV BACmid containing a deletion of the 79-nt ENE in PAN was generated to assess the effects of the ENE during viral replication. Our studies demonstrated that the ENE is not required for viral DNA synthesis, lytic gene expression, or production of infectious virus. Although the ENE is not required for viral replication, we found that the ENE functions to retain PAN in the nucleus and the absence of the ENE results in an increased accumulation of PAN in the cytoplasm. Furthermore, ORF59, LANA, ORF57, H1.4, and H2A still retain the ability to bind to PAN in the absence of the ENE. Together, our data highlights how the ENE affects the nuclear retention of PAN but ultimately does not play an essential role during lytic replication. Our data suggests that PAN may have other functional domains apart from the ENE.

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6.5

Cellular kinases modulate KSHV latency maintenance

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Developing specific therapies for KS is challenging as few virus-specific targets exist in latently infected KS tumors. Therefore, identifying effective pharmacological agents for KS treatment could be enhanced by a better understanding of cellular mediators of KSHV latency. Kinases are important regulatory proteins that control many host and viral processes, including oncogenesis. For this reason, kinase inhibitors are among the most successful targeted cancer therapies, with >50 FDA-approved drugs and hundreds in clinical development. Elucidating kinase-dependent signaling and pharmacological agents effective in regulating KSHV latency maintenance would provide critical insights into KSHV latency dependency factors and new potential therapeutic approaches for KS.To better understand the roles of kinases in KSHV latency, we applied the KiR (Kinome Regularization) analysis to a poly-pharmacology-based functional screen of the human kinome in iSLK cells latently infected with a KSHV recombinant containing a lytic replication reporter gene. This analysis utilized large-scale drug-target profiling data, regularized regression, and broadly selective chemical tool compounds to pinpoint specific kinases and associated networks governing the KSHV latent-to-lytic replication switch and to identify kinase inhibitors predicted to regulate KSHV latency maintenance. This kinome screening approach identified several kinases not previously linked to KSHV latency, as well as others that have been reported in other screens, including EGFR, MAPK14, and MAPK12 kinases. Additionally, based on this screen, we predict that several FDA-approved kinase inhibitors that are known to restrict virus replication of other herpesviruses also regulate the maintenance of KSHV latency. Current studies are underway to validate the predicted kinases and kinase inhibitors in regulating KSHV latency maintenance. Together, these findings will inform on KSHV latency dependency factors and therapeutic design for KS.

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6.6

Dual gRNA directed CRISPR/Cas9 Targeting of ORF73 Eradicates KSHV Genomes and Virion Production

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Maintenance of Kaposi's Sarcoma-associated Herpesvirus (KSHV) infection depends on the expression of ORF73, which encodes latency-associated nuclear antigen (LANA). During the KSHV life cycle, LANA plays several regulatory roles but also serves a structural function, tethering copies (often >100/cell) of the viral episome to host chromatin. This tethering is crucial for episomal maintenance during cell division and persistence of infection in the host. We hypothesized that parallel targeting of several regions of ORF73 using CRISPR/Cas9 in combination with multiple guide (g)RNAs, would lead to knock down of LANA expression and potential elimination of the viral genome. Employing lentiviral vectors encoding both CRISPR/Cas9 and a series of ORF73-targeted gRNAs, we identified two gRNAs that, together, were sufficient to maximally reduce levels of LANA in both immunofluorescence (IF) and western blot analyses. The first gRNA, previously described by Charles Wood and colleagues, targets a region toward the 5' end of ORF73, and the second targets a repeat element closer to the 3' end. The efficacy of the knockout was evident in multiple KSHV-positive cell lines. By 28 days, under selection, average LANA dot numbers per cell dropped by 99.7% in SLKp, 97.3% in L1T2, and 84.3% in iSLK-BAC16 cells without affecting viability. In contrast, identical treatment led to cell death in primary effusion lymphoma (PEL) lines, though as early as 4 days post-transduction, viable cells already showed a 45 and 66% drop in LANA dots, in BC-3 and BCBL-1 cells, respectively. These data suggest that the loss of viral genomes may underlie the PEL cell death. Remarkably, qPCR revealed that dual gRNAs also led to a near total loss (>97%) of intracellular KSHV DNA in KSHV-independent SLKp cells and nearly complete abrogation (>99%) of infectious virion production from induced iSLK-BAC16 cells. Combined, our findings suggest that efficient targeting of LANA, possibly via a CRISPR/Cas9-based method, could represent a future therapeutic approach to KSHV-associated tumors.

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Kaposi's Sarcoma Herpesvirus
(KSHV)
& Related Agents

Session VII:

Virus-Host Interactions-III

11:30am-1:00pm

7.1

Kaposi's Sarcoma-Associated Herpesvirus-Encoded circRNAs are Expressed in Infected Tumor Tissues and Are Incorporated into Virions

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Kaposi's sarcoma-associated herpesvirus (KSHV) has recently been found to generate circular RNAs (circRNAs) from several KSHV genes, most abundantly from K10 (viral interferon regulatory factor 4 [vIRF4]), K7.3, and polyadenylated nuclear (PAN) RNA. To define expression of these circRNAs, KSHV-infected cell lines, patient tissues, and purified virions were examined. KSHV circRNA expression was universally detected in tests of six primary effusion lymphoma (PEL) cell lines but ranged from low-level expression in BC-1 cells dually infected with tightly latent KSHV and Epstein-Barr virus to abundant expression in KSHV-only BCBL-1 cells with spontaneous virus production. Generally, the PAN/K7.3 locus broadly and bidirectionally generated circRNA levels that paralleled the corresponding linear RNA levels. However, RNA corresponding to a particular KSHV circularization site (circ-vIRF4) was minimally induced, despite linear vIRF4 RNA being activated by virus induction. *In situ* hybridization showed abundant circ-vIRF4 in noninduced PEL cells. All three KSHV circRNAs were isolated as nuclease-protected forms from gradient-purified virions collected from BrK.219 cells infected with a KSHV molecular clone. For circ-vIRF4, the fully processed form that is exported to the cytoplasm was incorporated into virus particles but the nuclear, intron-retaining form was not. The half-life of circ-vIRF4 was twice as long as that of its linear counterpart. The KSHV circRNAs could be detected at a higher rate than their corresponding linear counterparts by *in situ* hybridization in archival tissues and by reverse transcription-PCR (RT-PCR) in sera stored for over 25 years. In summary, KSHV circRNAs are expressed in infection-associated diseases, can be regulated depending on virus life cycle, and are incorporated into viral particles for preformed delivery, suggesting a potential function in early infection.

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7.2

Functional Analysis of vIRF4 Circular RNA in Kaposi's Sarcoma-Associated Herpesvirus

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Circular RNA (circRNA) is a class of RNAs that are single-stranded and form a closed structure via backsplicing, a process that covalently joins the 5' and 3' ends of exons. CircRNAs are relatively stable, resistant to exonucleases, and believed to be implicated in gene regulation and diseases, including multiple types of cancers. Recent studies using RNase R-treated RNAs revealed the circRNAome of Epstein Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), as well as interactions between KSHV and host circRNA. The KSHV viral interferon regulatory factor 4 (vIRF4) region expresses a protein and two circRNA isoforms with high expression in KSHV tumors, suggesting that circ-vIRF4 may contribute to KSHV pathogenesis and/or tumorigenesis. To characterize the function of circ-vIRF4, a KSHV mutant lacking the splice donor site (Δcirc-vIRF4) was generated in the bacmid BAC16. RT-PCR of Δcirc-vIRF4-infected iSLK cells shows that wild type isoforms are not detectable and cloning of products from Δcirc-vIRF4 suggests that alternative backsplice sites are used to express novel vIRF4 circRNAs. An initial gene expression analysis did not reveal significant expression changes of KSHV LANA, RTA, and ORF19 in Δcirc-vIRF4, but an RNA-seq analysis comparing WT- and Δcirc-vIRF4-infected iSLK cells during lytic replication demonstrated differential expression of host and viral genes. KSHV gene expression was generally downregulated, with some of the top downregulated genes belonging to the immediate early category of lytic genes. Gene ontology analysis of the top 50 differentially expressed host genes indicated roles in signal transduction, cell cycle, and apoptosis. These results suggest that a necessity exists for expressing circRNA from the vIRF4 locus and a potential role for circ-vIRF4 in regulating lytic gene expression. Work is ongoing to validate and study genes of interest from the gene expression analysis, as well as investigate potential differential gene expression in latently infected cells.

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7.3

Kaposi's sarcoma-associated herpesvirus induces spermidine and eIF5A hypusination for viral persistency via translation control of LANA

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Oncogenic viruses hijack host metabolism to induce tumorigenesis. One of the key cellular metabolites involved in tumorigenesis is polyamines, which are small positively charged molecules that are essential for fundamental processes of cell growth and survival. While polyamine is tightly regulated in normal cells, polyamine metabolism dysfunction and increased intracellular polyamine have frequently been observed in cancer cells. Here, we find that Kaposi's sarcoma-associated herpesvirus (KSHV), the causative agent of Kaposi's sarcoma and primary effusion lymphoma, upregulates the host polyamine metabolism, specifically spermidine, in 3D culture and 2D *de novo* infection conditions. KSHV regulates the expression of ornithine decarboxylase (ODC) and spermidine/spermine-N-acetyltransferase (SAT) enzymes that are essential for polyamine metabolism. Consequentially, increased spermidine is utilized for eukaryotic initiation factor 5A (eIF5A) hypusination that is crucial for translation of poly-proline containing protein, latency-associated nuclear antigen (LANA), a major KSHV latent protein. Inhibition of polyamine synthesis and eIF5A hypusination hinders the maintenance of viral episome and proliferation of KSHV-infected cells, which could be recovered by spermidine supplement. These results suggest targeting polyamine metabolism and eIF5A hypusination as a potential therapy for KSHV-associated tumor.

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7.4

Polyamine biosynthesis and eIF5A hypusination are modulated by the DNA tumor virus KSHV and promote KSHV viral infection.

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Polyamines are critical metabolites involved in various cellular processes and often dysregulated in cancers. Kaposi's sarcoma-associated Herpesvirus (KSHV), a defined human oncogenic virus, leads to profound alterations of host metabolic landscape to favor development of KSHV-associated malignancies. In our studies, we identified that polyamine biosynthesis and eIF5A hypusination are dynamically regulated by KSHV infection through modulation of key enzymes of these pathways. In return these metabolic pathways are required for both KSHV lytic switch and *de novo* infection. Further analysis unraveled that translation of critical KSHV latent and lytic proteins (LANA, RTA) depends on hypusinated-eIF5A. We also demonstrated that KSHV infection can be efficiently and specifically suppressed by inhibitors targeting these pathways. Collectively, our results illustrated that the dynamic and profound interaction of a DNA tumor virus (KSHV) with host polyamine biosynthesis and eIF5A hypusination pathways promote viral propagation and oncogenesis, thus defining new therapeutic targets to treat KSHV-associated malignancies.

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7.5

The m^{6}A epitranscriptomic landscape of polyadenylated nuclear (PAN) RNA

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Polyadenylated nuclear (PAN) RNA is a non-coding transcript involved in regulating Kaposi's sarcoma-associated herpesvirus (KSHV) gene expression, modulation of host immune response, and nuclear export of late viral mRNAs.

We have previously shown that PAN has a dynamic secondary structure and protein binding profiles, that can be influenced by the epitranscriptomic modifications to RNA[1].

N6-methyladenosine (m^{6}A) is one of the most abundant signatures found in viral and virus-encoded RNAs. Here, we addressed the dynamics of m^{6}A landscape of PAN RNA expressed during latent and lytic stages of KSHV replication by applying the 2nd and 3rd (Nanopore) generation RNA sequencing analyses. We showed that PAN RNA is extensively modified at the late lytic stages of KSHV replication, in contrast to most of its epitranscriptome. Using a newly developed method, termed selenium-modified deoxythymidine triphosphates (SedTTP)-RT and ligation-assisted PCR analysis of m^{6}A (SLAP), we determined the fraction of modification at each site. RNA antisense purification with mass spectrometry and immunoblotting, allowed identifying the m^{6}A readers, writers, and erasers that facilitate the modification of PAN RNA. By performing SHAPE-MaP RNA structure probing of PAN expressed in BCBL-1 cells with ablated expression of these enzymes, we showed that m^{6}A influences not only the local but also global folding of PAN RNA, leading to significant structural alternations within the expression and nuclear retention element (ENE), which is adjacent to two m^{6}A. To our knowledge, this work represents the first comprehensive overview of the dynamic interplay that takes place between the cellular epitranscriptomic machinery and a specific viral RNA in the context of infected cells.

[1] Sztuba-Solinska J, Rausch JW, Smith R, Miller JT, Whitby D, Le Grice SFJ. Nucleic Acids Res. 2017, 45(11):6805-6821.

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7.6

The Host Strikes Back: C19ORF66 restricts KSHV Lytic reactivation by Inducing KSHV Viral RNA Decay

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During lytic reactivation, KSHV seizes control of cellular gene expression by inducing a massive global RNA decay event termed “host-shutoff”. For KSHV, host-shutoff is orchestrated from the cytoplasm by SOX, a viral endoribonuclease, which degrades the vast majority of host mRNA (>70%). This viral takeover strategy is believed to rapidly co-opt host resources for viral replication while simultaneously downregulating the host anti-viral response. However, we and others have shown that select host transcripts still actively evade cleavage. Recently, we demonstrated that one such transcript, C19ORF66 (C19), is actively protected from cleavage and acts a potent anti-KSHV regulator, restricting multiple stages of KSHV lytic replication. Several emerging lines of evidence have shown that C19 restricts replication of multiple DNA, RNA, and retro- viruses, often by directly triggering viral RNA decay or by inhibiting critical viral strategies such as ribosomal frameshifting. We are currently investigating whether these strategies are also in place during KSHV infection. Here, show that overexpression of C19 stringently inhibits expression of critical viral early genes, most notably, the latent-to-lytic master switch protein, KSHV’s ORF50 (RTA). Using a combination of Actinomycin D and RNA Immunoprecipitation assays, we have found that C19 directly induces RTA mRNA decay and are currently exploring whether this decay is triggered by C19 directly binding RTA mRNA. Using mass spectrometry, we have also uncovered several host and KSHV proteins that interact with C19 including KSHV ORF57 and have constructed a C19 mutant library to map the interaction interface between C19, these protein interactors, and its target RNAs. By characterizing the interplay between C19 and KSHV, we hope, to better define the complex balance between KSHV and its host for control of cellular gene expression.

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Session VIII:

Virus Pathogenesis & Cancer

7:00am-8:30am

8.1

A Gammaherpesviral Tegument Protein Sequesters SMC Proteins and Inhibits the DNA Damage Response

Florian Full, Doris Jungnickl, Brigitte Scholz, Anna Großkopf[1], Alexander S. Hahn[1], Alexandra Herrmann, **Armin Ensser**

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Question – The rhadinovirus prototype herpesvirus saimiri (HVS), rhesus monkey rhadinovirus (RRV) and the closely related Kaposi's sarcoma-associated herpesvirus (KSHV, HHV8) share many key biological features and readily infect cells of both human and primate origin. Nuclear domain 10 (ND10) components like PML, SP100 and ATRX restrict herpesviral infection, and herpesviruses antagonize this restriction by a variety of strategies, including degradation or relocalization of ND10 proteins. **Methods and Results** – In order to elucidate the mechanism, cellular interaction partners of the KSHV tegument protein ORF75 were identified by coimmunoprecipitation (IP) or proximity dependent biotinylation (BioID) followed by mass spectrometry (MS). Among other candidates, MS identified several proteins containing Structural Maintenance of Chromosomes (SMC) domains as interaction partners for the viral protein. Expression of KSHV ORF75 either depleted the SMC protein or relocated it to ND10 domains. The necessary domains of KSHV ORF75 for interaction were mapped by localization and IP; we further detected ORF75 conjugation with Ub-domain containing modifiers. Notably, one identified host SMC protein is a known tumor suppressor, involved in heterochromatin formation and in DNA repair by NHEJ. Therefore, we studied the DNA damage response (DDR) in cells transfected with the viral tegument protein ORF75 after inflicting DNA damage with bleomycin. Several markers of DDR, namely phosphorylation of DNA-PKcs, ATM, H2AX, p53, and formation of damage induced foci of 53BP1 and other proteins of the pathways, were strongly reduced in cells expressing KSHV ORF75. **Conclusions** – While activation of DDR is a known feature of viral lytic replication, our data indicates that inhibition, possibly involving SMC proteins, may take place in the early phase of infection and latency establishment. Furthermore, DDR inhibition and cell cycle dysregulation may be contributing factors to KSHV oncogenesis.

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8.2

The Central Role of KSHV in the Pathogenesis of African Endemic and Epidemic Kaposi's Sarcoma

Salum J. Lidenge[1,*], Andrew V. Kossenkov[2], For Yue Tso[3], Jayamanna Wickramasinghe[3], Sara Privatt[3], Owen Ngalamika[4], John R. Ngowi[1], Julius Mwaiselage[1], Paul M. Lieberman[2], John T. West[3] and Charles Wood[3]

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OBJECTIVE: In sub-Saharan Africa, both endemic Kaposi's sarcoma (EnKS) and HIV co-infection-associated epidemic Kaposi's sarcoma (EpKS) remain prevalent neoplasms. While KSHV is clearly the etiologic agent of KS, the mechanisms underlying KS development are not fully understood in both forms of the disease. Immune dysfunction has been associated with EpKS development, but the precise nature of the defect remains unclear. Our data suggests that a direct role of HIV-1 in KS tumor maintenance is unlikely given the lack of HIV-1 gene expression in EpKS tumor tissue, but local or peripheral immune dysregulation may potentiate or accelerate tumor growth. How HIV-1 exerts an indirect impact on the EpKS tumor microenvironment is unknown. We hypothesized that distinct peripheral and local immune responses and gene expression patterns would differentiate EpKS from EnKS, thereby defining potential roles of HIV-1 in KS. **METHODS:** Analysis of plasma cytokine, immune cell infiltration of KS tumor, and RNA-seq followed by multiparameter bioinformatics analysis were conducted to compare immunological and transcriptomes from EnKS and EpKS lesions. **RESULTS:** Cytokine expression pattern and immune cell infiltration in KS tumors were strikingly similar between EnKS and EpKS. Gene expression profiles strongly correlated between EpKS and EnKS patients (Spearman $r=0.83$, $p<10^{-10}$). A subset of genes involved in tumorigenesis and inflammation/immune responses showed higher magnitude, but not unique dysregulation in EnKS compared to EpKS**. CONCLUSION:** The lack of significant differentials in cytokine expression and immune cell infiltration together with a trend toward higher magnitude of gene dysregulation in EnKS coupled with the absence of HIV-1 transcripts in EpKS may suggest an indirect or systemic effect of HIV-1 to promote and sustain KS tumorigenesis.

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8.3

KSHV lytic replication plays a vital role in virus-induced lymphomagenesis during EBV co-infection

Rieble Lisa[1], Caduff Nicole[1], Jung Jae J.[2], Grundhoff Adam[3], Münz Christian[1]

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Primary effusion lymphoma (PEL) is a KSHV-associated B cell lymphoma which is in 90% of tumors also positive for EBV. Recent advances in our laboratory showed that EBV and KSHV dual-infected NOD-scid $\gamma_c^{-/-}$ mice reconstituted with human immune system components (huNSG) support persistent KSHV infection. This model revealed that dual infection increases tumorigenesis and EBV lytic gene expression. Sequencing of ex vivo cultured dual-infected B cells and PEL cell lines displayed upregulation of KSHV lytic genes, leading to the hypothesis that these genes interact with EBV and host factors to drive the increased tumorigenesis. Our current study focuses on the replication and transcription activator RTA, that induces the expression of KSHV lytic genes and the switch to lytic replication. *In vitro* dual-infections using a KSHV RTA stop mutant virus showed that while RTA expression is not detrimental for persistent infection of human B cells, its loss decreases the growth potential of in vitro generated dual-infected B cell lines (EKCLs). RNA sequencing of dual-infected human B cells further allowed us to shed more light on the impact of RTA and KSHV lytic gene expression on the host and EBV genome and reveal affected cellular and viral pathways and processes. *In vivo* infection of huNSGs revealed that, in line with our *in vitro* data, RTA did not impact KSHV persistence. However, tumorigenesis was significantly decreased in mice infected with KSHV RTA stop mutant compared to mice infected with KSHV wildtype. Our study is the first to examine the effect of KSHV lytic gene expression in a dual infection setting close to the naturally occurring infection and PEL development and can extend our knowledge on the interaction of KSHV with host and EBV genes, revealing new treatment targets.

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8.4

Cellular FLIP Protects KSHV-Infected Lymphoma Cells from TRAIL-Independent TRAIL Receptor 1-Mediated Death Signaling

Neil Kuehnle, Mark Manzano, Eva Gottwein

Kaposi's sarcoma-associated herpesvirus (KSHV) causes primary effusion lymphoma (PEL). We have previously shown that cultured PEL cells require expression of the cellular FLICE inhibitory protein (cFLIP) for survival, although KSHV encodes a viral homolog of this protein (vFLIP). Cellular and viral FLIP proteins have many functions, including most importantly the inhibition of pro-apoptotic caspase 8 and modulation of NF-kB signaling. Here, we took several approaches to investigate the role of cFLIP and its potential redundancy with vFLIP in PEL cells. First, we generated PEL cell lines overexpressing FLIP proteins with differential characterized activities towards FLIP target pathways, i.e., the long and short isoforms of cFLIP, KSHV vFLIP, or two vFLIP proteins encoded by the poxvirus molluscum contagiosum virus. Our results indicate that only FLIP proteins that are strong caspase 8 inhibitors efficiently rescue loss of endogenous cFLIP activity in PEL cells. Thus, the function of cFLIP in PEL cells is largely distinct from the role of KSHV vFLIP. Next, we employed genome-wide CRISPR/Cas9 synthetic rescue screens to identify loss of function perturbations that can compensate for cFLIP knockout. Results from these screens and our validation experiments implicate the canonical cFLIP target caspase 8 and TRAIL receptor 1 (TRAILR-1 or TNFRSF10A) in promoting constitutive death signaling in PEL cells. However, to our surprise, we do not detect endogenous soluble or membrane-bound TRAIL in PEL cells. Our ongoing studies seek to identify the process responsible for this TRAIL-independent TRAILR-1 activity, by employing differential CRISPR/Cas9 essentiality screens in the context of cells lacking caspase 8. Preliminarily, results from these screens suggest that TRAILR-1 functions at the core of a previously uncharacterized cellular stress response that is active in cultured PEL cells. In sum, our work shows that PEL cells require cFLIP to inhibit constitutive TRAIL-independent TRAILR-1-mediated death signaling.

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8.5

AS1411 aptamer displays potent therapeutic potential in Kaposi's sarcoma-associated virus (KSHV)-associated Primary effusion lymphoma

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Primary effusion lymphoma (PEL) is a rare and aggressive tumor of hematopoietic and lymphoid tissues. Kaposi's sarcoma-associated herpesvirus (KSHV) infection is a risk factor for PEL development, and it is found predominantly in late-stage AIDS patients. PEL has a poor prognosis, and there is a critical need for antiviral drugs specifically targeting KSHV and have fewer adverse effects. A previous study from our lab (J Virol 94: e02177-19) suggested the therapeutic potential of an endogenous anti-inflammatory lipid mediator lipoxin A4 (LXA4) as one of the safe therapeutic molecules to block KSHV infection. Using mass spectrometry, we identified a multifunctional DNA/RNA-binding protein nucleolin (NCL) as one of the LXA4-interacting nuclear proteins. This motivated us to understand the role of nucleolin and its regulation by LXA4 during KSHV infection. Here, we identified that de novo KSHV infected endothelial cells, PEL cells, and human skin Kaposi's sarcoma (KS) tissue sections abundantly express nucleolin and phospho-nucleolin. This study targeted nucleolin with anti-nucleolin aptamer AS1411, which has been tested in clinical trials, in vitro, and in vivo models of many cancers. AS1411 is a 26-mer unmodified guanosine (G)-rich oligonucleotide [5'-d(GGT GGT GGT GGT TGT GGT GGT GGT GG)-3']. We found it efficacious in inducing cell death in KSHV infected PEL cell lines. AS1411 treatment or nucleolin silencing in KSHV infected cells reduced KSHV latent (ORF73), increased lytic (ORF50) gene expression, and lytic reactivation. Nucleolin silencing of KSHV infected PEL cells (BCBL-1 and BC-3) downregulated survival kinases of the AKT pathway. Interestingly, we identified the synergistic effect of AS1411 and LXA4 in inducing PEL cell death, inhibiting PEL cell proliferation without much KSHV lytic replication. This synergistic effect was due to LXA4 mediated downregulation of nucleolin phosphorylation.

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8.6

Viral glutamine amidotransferase (vGAT) activates phosphoribosyl-formylglycinamidine synthetase (PFAS) to fuel *de novo* purine synthesis and tumorigenesis

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi's sarcoma, pleural effusion lymphoma and multicentric Castleman's disease. Though viruses are known to activate cellular metabolism to promote their infection, the molecular mechanism by which viruses do so is not well understood. We previously reported that vGAT, a viral pseudoenzyme of cellular PFAS, recruits PFAS to deamidate RIG-I and block antiviral cytokine production. PFAS serves as a scaffold of the purinosome, a metabolic machinery responsible for the *de novo* purine biosynthesis. Thus, we determined whether KSHV vGAT influences the *de novo* purine synthesis which may underpin KSHV-associated malignancies. Steady state metabolite profile reveals that vGAT increases the intracellular intermediates of de novo purine pathway, such as IMP, inosine, hypoxathine and guanosine in human oral keratinocytes (HOK) cells, suggesting that vGAT elevates the *de novo* purine synthesis. Indeed, flux analysis using U-^{13}C-labeled glucose showed that vGAT increases the carbon incorporation into purine. Additionally, vGAT binds to purine biosynthetic enzymes, including PFAS and phosphoribosyl pyrophosphate amidotransferase (PPAT). Interestingly, NIH3T3 cells expressing vGAT formed tumors in nude mice, implicating its potential contribution to KSHV-associated malignancies. Ongoing work is directed to determine the mechanism of action of vGAT in regulating *de novo* purine synthesis and its potential role in KSHV pathogenesis.

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KSHV 2021 Virtual Meeting on
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Session IX:

Virus-Host Interactions-IV

9:00am-10:30am

9.1

Exosome-encased Nucleic Acids Scaffold Chemotherapeutic Agents for Targeted Tumor Delivery

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Extracellular vesicles (EVs) and exosomes play a pivotal role in tumor growth and metastasis. KSHV repackages EVs from infected cells with viral miRNAs and sends these modified EVs, which we term KSHV-EVs, to neighboring, naïve cells, sensitizing them to a pro-tumor phenotype without triggering innate immune signaling cascades. Here, we reverse this phenotype through the loading of chemotherapeutic drugs into KSHV-EVs, converting their pro-tumor/pro-angiogenic phenotype into an anti-tumor phenotype *in vivo*. Drug-concentration was significantly higher in EVs than in currently-used liposome formulation as retention was facilitated by the presence of the high concentration of miRNAs inside the KSHV-EVs. By exploiting the targeting preferences of KSHV-EVs, chemotherapeutics can be directed to specifically poison the cells and the microenvironment that enable metastasis.

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9.2

Iron Metabolism and Ferroptosis Play a Role in Kaposi Sarcoma Herpesvirus Pathogenesis and Represent Therapeutic Targets

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Ferroptosis is a novel form of regulated cell death driven by excessive iron-dependent lipid peroxidation and an inadequate lipophilic antioxidant response. Cancer cells require high levels of labile iron to fuel growth and resist ferroptosis via activation of two independent antioxidant pathways. The glutathione (GSH)-dependent pathway is triggered by system x_c^- (xCT), which imports cystine for GSH synthesis, and relies ultimately on the enzyme GPX4 using GSH to reduce lipid peroxides. The second depends on the recently identified ferroptosis suppressor protein 1 (FSP1), which catalyzes the regeneration of CoQ10 to act as a radical-trapping antioxidant. We have previously determined that Kaposi sarcoma herpesvirus (KSHV) manipulates host iron metabolism to promote iron acquisition and an iron-responsive growth phenotype. The goal of the current study is to determine (i) how KSHV-infected cells resist iron-catalyzed ferroptosis, and (ii) if perturbation of ferroptosis suppressor pathways selectively kills infected cells. Using lymphatic endothelial cells (LEC) *de novo*-infected with KSHV-BAC16 as an *in vitro* KS model, expression and activity of key components of the GSH-dependent and CoQ-dependent ferroptosis suppressor pathways was examined. Susceptibility to ferroptosis was evaluated using selective inducers or inhibitors and measured via cell viability and lipid peroxidation assays. We find that KSHV significantly deregulates the GSH-dependent ferroptosis suppressor pathway such that infected LEC become dependent on xCT for survival and are selectively killed by xCT inhibition. Notably, KSHV also upregulates FSP1, identifying a second potential ferroptosis escape mechanism in infected cells. We are currently exploring the consequences of targeting this novel suppressor pathway. We have identified unique vulnerabilities in KSHV-infected cells that reflect the delicate pro/antioxidant balance required to facilitate tumorigenesis. Activation of ferroptotic death in tumors cells represents an evolving area of cancer research, and our work suggests that selective induction of ferroptosis in KSHV-infected cells represents a promising anti-KS strategy.

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9.3

KSHV Infection of Lymphatic Endothelial Precursor Cells

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The main proliferating cell of KS tumors is the spindle cell, a cell that has markers of lymphatic and blood endothelium as well as mesenchymal cell markers. Though it has been noted that KS does not occur in sites in the body devoid of lymphatics, the true origin of spindle cells is unknown. A few groups have proposed that endothelial precursor cells, cells which have endothelial cell markers but transit in the blood and are known to traffic to sites of angiogenesis, could be the source of spindle cells. Furthermore, a study showed that in solid-organ transplant recipients with KSHV+ donors, when KS formed on the lower extremities, the tumor cells were donor derived cells, not newly infected cells of the recipient, indicating that the precursor to spindle cells are able to transit in the body. We previously isolated endothelial colony forming cells (ECFCs), a type of isolation of endothelial precursor cells, and found that there were actually two types of ECFCs, ones that expressed markers of blood endothelium and ones that expressed markers of lymphatic endothelium. Here we examined KSHV infection of both blood and lymphatic ECFCs. We found that similar to adult and neonatal blood and lymphatic endothelial cells, when infected at low MOI the lymphatic ECFCs are infected to significantly higher levels than blood ECFCs and while the blood ECFCs lose the viral episome during passage, lymphatic ECFCs maintain KSHV infection over longer passage. KSHV infection slightly decreases the proliferation of both cell types but does not have a significant effect on the ability of both cell types to form capillary-like structures in a three-dimensional Matrigel matrix. Uninfected and KSHV infected blood ECFCs and uninfected lymphatic ECFCs were unable to proliferate in soft agar. However, over the course of four weeks, the KSHV infected lymphatic ECFCs grew to small multicellular colonies in soft agar. While small colonies were only ever identified in the KSHV infected lymphatic ECFCs, the colonies were limited in cell number and generally did not expand to larger colonies as fully transformed cell lines could. This data demonstrates that KSHV appears to provide a limited transformation phenotype to infected lymphatic but not blood ECFCs promoting the possibility that lymphatic ECFCs could be the source of spindle cells.

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9.4

Gene Expression Changes That Define the Antiviral Activity of 25-Hydroxycholesterol Against KSHV and EBV Infections

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A cholesterol derivative, 25-hydroxycholesterol (25HC), has been demonstrated to inhibit infections from widely different bacteria and viruses, including SARS-CoV2. However, its mechanism of activity is still under study. To characterize the gene regulatory pathways triggered by 25HC, we performed RNA sequencing (RNA-Seq) of both EBV (Epstein-Barr Virus/HHV-4)-infected B cells and KSHV (Kaposi's Sarcoma Herpesvirus/HHV-8)-infected primary endothelial cells that have been treated with 25HC. We looked at the gene expression changes in the host and virus after 25HC treatment to help define its antiviral activity in the context of these human herpesviruses. We found that 25HC resulted in global downregulation of KSHV viral genes, with greater impact on lytic genes compared to latent genes. In contrast, we found that only a subset of EBV viral genes are downregulated, including noncoding RNAs EBER1 (EBV-encoded small RNA 1) and EBER2. RPMS1, a lncRNA (long noncoding RNA) generated as an alternatively spliced transcript from the BART (*Bam*-HI A rightward transcripts) region, which also encodes for the BART miRNAs, was also significantly reduced by 25HC. Interestingly, LMP1 (latent membrane protein 1), which is the main EBV oncoprotein that drives proliferation and inhibits apoptosis in infected B cells, was also downregulated by 25HC. Looking at the gene expression changes in the host, we saw that 25HC treatment induced a type I interferon (IFN) response, including interferon-stimulated genes and several inflammatory cytokines (CXCL8, IL1α). As for the broadly antiviral effect of 25HC, we found that 25HC-induced genes in our KSHV data set corresponded with hits for antiviral genes found in other screens for unrelated viruses. Likewise, 25HC-downregulated genes overlapped with a list of host-dependency factors identified in screens for other viruses. Together, these results bring us closer to understanding how a modified form of cholesterol works against several pathogens.

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9.5

BiP Activity Is Critical for KSHV Reactivation and Survival of Latently Infected B Cells

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Productive KSHV infection involves the translation, folding, and modification of abundant viral proteins in the Endoplasmic Reticulum (ER). The heightened demand for ER function can disrupt ER homeostasis and activate the Unfolded Protein Response (UPR), a collection of stress signaling pathways in charge of restoring ER homeostasis. Three ER-resident sensors/transducers, the kinase/RNase IRE1, the kinase PERK, and the membrane-bound transcription factor ATF6, govern the UPR. Their activation culminates in gene regulatory programs that promote adaptation or cause apoptosis if homeostasis is not restored. GRP78/BiP is the most abundant ER chaperone and a major regulator of IRE1 and PERK. Its expression is mainly regulated at the transcriptional level by ATF6 in response to ER stress. Our results show that lytic reactivation of KSHV in iSLK-219 cells leads to the ATF6-independent accumulation of GRP78/BiP. The upregulation of GRP78/BiP is critical during the lytic cycle. Treatment of KSHV-infected epithelial (iSLK-219) or B-cells (BCBL-1) with HA15, a potent GRP78/BiP inhibitor, reduces late-lytic gene expression and infectivity. Moreover, our results suggest that BiP activity may have an essential role in the survival of BCBL-1 cells as treatment with HA15 leads to cell death in this cell line. Importantly, HA15 treatment is not cytotoxic in uninfected B-cells, primary endothelial cells, or epithelial cells. Our results indicate that targeting BiP is a promising strategy to prevent KSHV lytic replication and may offer an attractive target for the development of therapeutics for the treatment of KSHV related malignancies.

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9.6

SARS-CoV-2 proteins and anti-COVID-19 drugs induce lytic reactivation of KSHV

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Abstract: An outbreak of the novel coronavirus SARS-CoV-2, the causative agent of Coronavirus Disease-2019 (COVID-19), a respiratory disease, has infected over 120,000,000 people since the end of 2019, killed over 2,600,000, and caused worldwide social and economic disruption. Because the mechanisms of SARS-CoV-2 infection of host cells and its pathogenesis remain largely unclear, there are currently no antiviral drugs with proven efficacy. Besides severe respiratory and systematic symptoms, several comorbidities increase risk of fatal disease outcome. Therefore, it is required to investigate the impacts of COVID-19 on pre-existing diseases of patients, such as cancer and other infectious diseases. Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent of several human cancers, such as Kaposi's sarcoma (KS) and Primary effusion lymphoma (PEL), mostly seen in immunosuppressed patients especially people with HIV infection. In the current study, we for the first time, report that SARS-CoV-2 encoded spike (S) and nucleocapsid (N) proteins and some currently used anti-COVID-19 drugs (especially Azithromycin and Nafamostat mesylate) are able to induce lytic reactivation of KSHV from virus infected normal and tumor cells, through manipulation of intracellular signaling pathways. In addition, we found the obvious upregulation of ACE2 expression, the major receptor of SARS-CoV-2, in AIDS-KS tissues, indicating that KSHV may potentially increase SARS-CoV-2 co-infection. Together, our data indicate that those KSHV+ patients especially in endemic areas exposure to COVID-19 or undergoing the treatment may have increased risks to develop these virus-associated cancers, even after they have fully recovered from COVID-19.

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KSHV 2021 Virtual Meeting on
Kaposi's Sarcoma Herpesvirus
(KSHV)
& Related Agents

Session X:

Therapeutics

11:00am-12:30pm

10.1

High levels of LINE-1 transposable elements expressed in Kaposi's sarcoma-associated herpesvirus-related primary effusion lymphoma contribute to their proliferation

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Kaposi's sarcoma associated herpesvirus (KSHV, HHV-8) is a gamma herpesvirus associated with several human malignancies. Transposable elements (TEs) are ubiquitous in eukaryotic genomes, occupying about 45% of the human genome. TEs have been linked with a variety of disorders and malignancies, though the precise nature of their contribution to many of them has yet to be elucidated. Global transcriptome analysis for differentially expressed TEs in KSHV-associated primary effusion lymphoma (PEL) cells (BCBL1 and BC3) revealed large number of differentially expressed TEs. These differentially expressed TEs include LTR transposons, Long Interspersed Nuclear Elements (LINEs), and Short Interspersed Nuclear Elements (SINEs). Further analysis of LINE-1 (L1) elements revealed expression up-regulation, hypo-methylation and transition into open chromatin in PEL. In agreement with high L1 expression, PEL cells express ORF1 protein and possess high Reverse Transcriptase (RT)-activity. Interestingly, inhibition of this RT-activity suppressed PEL cell growth. Collectively, we identified high expression of TEs, and specifically of L1 as a critical component in the proliferation of PEL cells. This observation is relevant for the treatment of KSHV-associated malignancies since they often develop in AIDS patients that are treated with HIV RT inhibitors, some also are potent inhibitors for L1 RT activity.

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10.2

Primary effusion lymphoma enhancer connectome links super-enhancers to dependency factors

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Primary effusion lymphoma (PEL) has a very poor prognosis. To evaluate the contributions of transcription enhancers/promoters to PEL cell growth and survival, H3K27ac HiChIP was used to generate the PEL enhancer connectome, linking enhancers and promoters in both Kaposi's sarcoma-associated herpesvirus (KSHV) singly infected and KSHV/Epstein-Barr virus (EBV) coinfected PEL cell lines, genome-wide. More than 8000 genomic interactions were identified in these PEL cell lines. Incorporating HiChIP data with H3K27ac ChIP-seq data, interactions between enhancers/enhancers, enhancers/promoters, and promoters/promoters were identified. HiChIP linked PEL super-enhancers (SEs) to PEL dependency factors MYC, IRF4, MCL1, CCND2, MDM2, and CFLAR. CRISPR knock out of MEF2C and IRF4 significantly reduced MYC and IRF4 SEs H3K27ac signal. Knock out also reduced MYC and IRF4 expression. CRSIPRi perturbation of these SEs by tethering transcription repressors to SEs significantly reduced SE target gene expression and reduced PEL cell growth. These data provided new insight into PEL molecular pathogenesis.

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10.3

XPO1 is a vulnerable target of Kaposi's sarcoma and primary effusion lymphoma, and mediates innate immune response during KSHV primary infection

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While the incidence of Kaposi's sarcoma (KS) has been significantly reduced following the advent of anti-retroviral therapy, a substantial portion of AIDS patients continue to develop KS in Western countries. In some parts of Africa, KS has emerged as the most common cancer. Hence, it is of urgent need to identify novel therapeutic targets and agents. By performing genome-wide clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) screening, we have previously identified cancer-additive genes of Kaposi's sarcoma-associated herpesvirus (KSHV)-induced cellular transformation. Among the identified targets, we showed that exportin 1 (XPO1) was critical for KSHV-induced cellular transformation. Both XPO1 inhibitor KPT-8602 and small interfering RNA (siRNA) knockdown inhibited cell proliferation and induced cell death in KSHV-induced KS-like cells and primary effusion lymphoma (PEL) cell lines. Mechanistically, XPO1 inhibition induced relocalization of autophagy adaptor protein p62 (SQSTM1) in promyelocytic leukemia (PML) nuclear bodies, which subsequently activated p53 and caused cell cycle arrest and apoptosis. In a xenograft PEL model, KPT-8602 treatment not only effectively inhibited the initiation and progression of PEL lymphomas but also induced regression of grown PEL lymphomas. To determine the role of XPO1 in KSHV infection, we employed a model of KSHV infection of primary human umbilical vein endothelial (HUVEC) cells. XPO1 inhibition induced retention of p62 in the nucleus, resulting in the activation of TBK1 and IRF3. As a result, nuclear accumulation of p62 increased the expression of innate immune-related genes including IRF7, ISG15, IFIT1, IFIT2, and IFIT3, leading to reduced KSHV lytic replication. Taken together, we have demonstrated that XPO1 is not only a vulnerable target of KSHV-induced cancers, but also a potential therapeutic target for KSHV infection and replication.

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10.4

Metabolic roles of IKKepsilon in KSHV induced tumor growth

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IκB kinase (IKK) epsilon is a member of non-canonical IKKs that regulate signal transduction underpinning inflammation and cancer. IKKepsilon was shown to be overexpressed in diverse tumors and its roles have been extensively studied in breast and colon cancers. We previously reported that IKKepsilon is required for KSHV GPCR-induced cellular transformation relevant to KS. In this study, we are investigating the roles of IKKepsilon in KSHV-positive primary effusion lymphoma (PELs). Our initial results showed that KSHV+ PEL cells require IKKepsilon for their growth and proliferation. To further understand the molecular action of IKKepsilon, we employed high throughput phospho-proteomics to profile the phosphoproteins that are impacted by IKKepsilon-depletion. IKKepsilon-depletion induced distinct phosphorylation landscape with changes of phosphorylated proteins enriched in cell cycle, mTOR pathway and nucleotide metabolism. Interestingly, significant changes in phosphorylation of glutamine amidotransferases in nucleotide metabolism were detected, such as CAD, CTPS1, PFAS and GMPS. Glutamine amidotransferases have intrinsic activities to deamidate key signaling proteins and alter fundamental biological processes. Finally, we showed that IKKepsilon physically interacted with GMPS, CAD and PFAS and nucleotide metabolism significantly downregulated by IKKepsilon depletion. Current research is geared to examine the molecular mechanism that IKKepsilon regulates nucleotide synthesis and cell cycle via cellular glutamine amidotransferases. Our work will reveal new roles of IKKepsilon in KSHV persistent infection and foster potential crosstalk between protein kinases and deamidases.

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10.5

Inflammasome activation in patients with KSHV-associated disorders

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Kaposi sarcoma herpesvirus (KSHV) causes several disorders: Kaposi sarcoma (KS), primary effusion lymphoma (PEL), a plasmablastic form of multicentric Castleman disease (MCD), and most recently inflammatory cytokine syndrome (KICS), which may occur alone or concurrently in the same patient. KSHV-associated disorders (KAD), which can occur among people living with HIV, are characterized by elevated circulating levels of inflammatory mediators, resulting in systemic symptoms that can result in morbidity and mortality. Activation of the inflammasome, which leads to pro-inflammatory cytokines such as IL-1β and IL-18 via active caspase-1, has not been assessed in patients with KAD. Peripheral blood mononuclear cells (PBMCs) from 9 HIV-negative control participants (HCs) and 33 cisgender male participants with HIV infection and KAD [PEL (5), MCD (9), KS (6), KICS (6) or overlap of these conditions (7)] were incubated with a probe to assess active caspase-1 (FLICA) followed by staining for ASC (apoptosis associated speck like protein containing CARD) specks in peripheral monocytes to detect inflammasomes with active caspase-1. T-test and Wilcoxon rank sum tests were used to assess differences in speck formation and plasma cytokine levels between HC and KAD participants. KAD participants had higher IL-18 plasma levels than HCs (median: 2959 vs. 794 pg/ml, p=0.0009), indicating that KAD are accompanied by systemic inflammasome activation. We found higher levels of monocytes demonstrating spontaneous $FLICA^{+}$ASC-speck formation in participants with KAD compared to HCs (KAD: 89,546 cells/mL, 4.60% *vs* HCs: 30,965 cells/mL, 1.39%, p=0.0002 and p <0.0001). Overall, as compared to participants with one KAD, those with more than one KAD did not have significantly different monocytes with $FLICA^{+}$ASC-specks/mL (p=0.09). Our data suggest that activation of the inflammasome in circulating blood monocytes contributes to the pathogenesis of KSHV-associated disorders and could potentially represent a target for host-directed therapy against these diseases.

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10.6

Targeting Metabolic Vulnerabilities in Primary Effusion Lymphoma Using the Novel Nucleoside Analog (6-ETI)

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Nucleoside analogs are widely used in the clinic for the treatment of hematological malignancies. These drugs, while very useful, have limited efficacies and patients frequently develop resistance. Thus, identifying novel therapies with predictive biomarkers to personalize therapy is crucial. We previously reported the discovery of the nucleoside analog 6-ethylthioinosine (6-ETI) as a potent and selective inhibitor of primary effusion lymphoma (PEL). PEL is an aggressive B-cell non-Hodgkin's lymphoma associated with KSHV infection, known to be refractory to conventional chemotherapy. Our studies demonstrated that 6-ETI is a pro-drug activated by adenosine kinase (ADK), an enzyme that is overexpressed in PEL cell lines and primary specimens. 6-ETI induced S phase arrest and inhibited DNA synthesis. RNA sequencing of *in vitro* generated PEL resistant clones and CRISPR knock out of ADK (ADK KO), respectively, indicated that mutations or loss of expression of ADK renders cells resistant to treatment. This data indicates that ADK expression can be used as a predictive biomarker of response to 6-ETI. Here, we elucidate the mechanism of action of this drug and further characterize the role of ADK in PEL. Investigating pathways that are differentially regulated in sensitive and resistant cells, we found that drug sensitivity was associated with AMPK activation and inhibition of PI3K/mTOR/p70S6K signaling. Integrated metabolic and transcriptomic profiling revealed that 6-ETI depletes wild-type PEL cells of their nucleotide pools accompanied by the downregulation of genes in purine and pyrimidine biosynthesis pathways, however; ADK KO resistant cells have reprogrammed to upregulate *de novo* pyrimidine biosynthesis pathway and p70S6K signaling. Purine metabolism is a critical mediator of 6-ETI-induced cytotoxicity since supplementation with adenine rescues wild type PEL, reverses p70S6K inhibition and restores DNA synthesis. Moreover, we found that 6-ETI synergizes with the pan PI3K inhibitor BKM120 highlighting nucleotide metabolism and PI3K/mTOR signaling as key therapeutic vulnerabilities targeted by 6-ETI.

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