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Istituto Europeo di Oncologia

7TH MEETING ON EMERGING ISSUES IN ONCOGENIC VIRUS RESEARCH

3-8 June 2025

ABSTRACT BOOK

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INVITED SPEAKERS

ORAL AND POSTER PRESENTERS

HOW TO MAKE AND SHARE VACCINE-STYLE BEER
Presenter: Christopher Buck

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BACKGROUND-AIM
Although early vaccines consisted of crude preparations of live pathogens delivered via oral, intranasal, or intradermal routes, safer modern vaccines tend to use killed pathogens or purified subunits that are injected intramuscularly. Although there has been a general dogma that oral vaccine delivery can only work with live pathogens, a recently developed oral cholera vaccine containing a recombinant toxin subunit is highly effective. We hypothesized that a subunit vaccine targeting the BK polyomavirus (BKV) major capsid protein (VP1) might be immunogenic when administered via oral, nasal, dermal, or rectal routes.
METHODS
BKV VLPs were initially produced using a baculovirus-based expression system.
RESULTS
Purified BKV VP1 virus-like particles (VLPs) administered intranasally or intradermally elicited average neutralizing antibody titers of roughly 900,000 and 7,500, respectively. Oral and rectal administration of purified VLPs did not induce detectable antibody responses. To test the idea that the immunogenicity of purified VLPs might be degraded by exposure to stomach acid or digestive enzymes, we administered VLPs protected within live brewer's yeast engineered to express BKV VP1. Mice that ate live yeast mixed with regular mouse chow developed average neutralizing titers above 10,000 - a level that has previously been shown to correlate with resistance to BKV-induced kidney disease in organ transplant patients.
CONCLUSIONS
The results open the door to the rapid development of vaccine-style beer that can be marketed with structure/function claims along the lines of "supports healthy immune system function," in compliance with the 1994 Dietary Supplement Health and Education Act. Plasmids will be shared under OpenMTA.

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ROLE OF RAB GTPASES IN HUMAN PAPILLOMAVIRUS ENTRY
Presenter: Jeongjoon Choi

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BACKGROUND-AIM
Rab GTPases play key roles in controlling intracellular vesicular transport, with their GTP-bound form typically supporting trafficking. Rab proteins are required for retrograde transport of human papillomaviruses (HPVs) from endosome to the trans-Golgi network (TGN), Golgi apparatus, and eventually nucleus during entry, but it remains largely unclear how HPV utilizes specific Rab protein(s) at distinct entry steps. The non-enveloped HPV capsid consists of the major (L1) and minor (L2) capsid proteins. The protein sorting complex retromer and a motor protein complex dynein associate with HPV and are implicated in HPV entry. Rab7 is critical for retromer-mediated endosome-to-TGN HPV trafficking.
METHODS
We investigated the role of Rab6a and Rab9a proteins in HPV entry through biological, biochemical, and imaging experiments in HeLa or 293 HEK cells infected with HPV pseudoviruses, as well as in vitro experiments with purified proteins and peptides.
RESULTS
Rab9a regulates HPV-retromer association independently of Rab7 by engaging with L2 early in entry, prior to the HPV-Rab7 interaction. Rab9a depletion dramatically increases HPV-retromer association but impairs endosome-to-TGN transport of HPV. Unlike cellular protein transport, GTP-bound Rab9a inhibits HPV infection whereas GDP-bound Rab9a promotes virus infection. Rab6a associates with HPV in the TGN at later entry stages, enabling HPV trafficking from the TGN to cis-Golgi. Rab6a supports HPV trafficking by promoting the association of HPV with dynein and a dynein adaptor BICD2 in the TGN. The HPV16 L2 C-terminus segment directly binds to GTP-Rab6a and BICD2 in vitro. Excess of either GTP-Rab6a or GDP-Rab6a inhibits HPV entry, suggesting that cycling between GDP-Rab6a and GTP-Rab6a is critical for infection.
CONCLUSIONS
Our findings reveal important features of the molecular basis of HPV trafficking during entry, including the discovery that HPV uses trafficking mechanisms that differ from those used by cellular proteins.

EARLY GENOME INSTABILITY UNDERPINS THE ORIGINS OF MERKEL CELL CARCINOMA
Presenter: James DeCaprio

Mackenzie Gilson1, Matheus de Melo Lobo2, Julia L. Schnabel2, Jingwei Cheng2,3, Thomas Frost2, Manisha Thakuria2, James A. DeCaprio2, Gabriel J. Starrett1
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BACKGROUND-AIM
Merkel cell carcinoma (MCC) is a rare, aggressive neuroendocrine tumor of the skin associated with either extensive UV mutagenesis or Merkel cell polyomavirus (MCPyV) infection. To date, most genomic characterization of MCC has used targeted sequencing of genes with a known role in cancer development and treatment. To better understand the origins and evolution of MCC, we performed whole genome sequencing (WGS) of 56 primary MCC with matching normal PBMC sequencing for 53 patients.
METHODS
Virus status was determined using the number of WGS reads mapping to the MCPyV genome normalized by the total number of bases with coverage of the integrated MCPyV and total human aligning reads.
RESULTS
Thirty-seven (66%) tumors were MCPyV-positive (MCCP) and nineteen (34%) were virus negative (MCCN). MCCN patients were older (median age: 78) and predominantly male (n=17, 89%) compared to MCCP (median age: 71; male: n=25, 68%). TP53 and RB1 were the most commonly mutated genes in MCC with 18 tumors harboring mutations in TP53 (1 MCCP, 17 MCCN), 17 with RB1 mutations, and 15 tumors harboring mutations in both genes (79% of MCCN). FAT1, NOTCH1, KMT2D, ASCM1 were also significantly mutated. MCPyV integration events were identified in all autonomic chromosomes. In one patient, two independent primary tumors had viral integration events on different chromosomes. Viral integration events were associated with microhomology-mediated end-joining, copy number repeats and translocation events. Average estimated telomere length for PBMCs were short (1.7 kbp, range: 1.2-2.8 kbp), consistent with values for patients with median age of 75. Both MCCP and MCCN tumors had shorter median telomere lengths (1.2 kbp, range: 0.7-4.0 kbp). UV-mediated mutation signatures (SBS7a/b) were observed in most MCCN tumors, with a low burden of UV-mediated mutations in half of MCCP tumors. Point mutations were timed relative to their allele frequencies and occurrence before or after copy number altering event adjusting by tumor purity. As expected, aging-related mutations were mostly seen as early clonal mutations in all tumors. In MCCN, UV mutations were present at all stages of tumor evolution. In MCCP, there was little evidence of substantial clonal expansion of point mutants after MCPyV integration, but rather subclonal mutations attributable to indirect UV damage, chemotherapeutics, and mutations of unknown processes.

CONCLUSIONS

Our study provides the most comprehensive molecular characterization of Merkel cell carcinoma identifying common and distinct molecular processes underpinning the origins and evolution of MCCN and MCCP. Both tumor types have substantially shortened telomeres which likely contribute to copy number variants and translocations, with some association with MCPyV integration. While MCCN tumors show evidence of substantial UV mutagenesis with multiple rounds of clonal expansion and evolution, MCCP tumors have fewer mutations with a greater proportion of subclonal variants suggesting that these provide little fitness advantage and selection pressure after MCPyV integration.

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MOUSE MODELS YIELD NEW INSIGHTS INTO VIRUS-POSITIVE MERKEL CELL CARCINOMA
Presenter: Andrzej Dlugosz

L. Syu 1, M. Verhaegen 1, P. Huang 1, H. Pinto 1, S. Mishra 1, H. Zhang 1, Z. Freeman 1, A. Dlugosz 1
1University of Michigan

BACKGROUND-AIM
The SLAP mouse model of virus-positive (VP) Merkel cell carcinoma (MCC) has many features in common with human VP MCC, including similar histology, protein marker expression, upregulation of TAg targets, and global as well as MCC-specific transcriptomes. SLAP mice also develop regional LN metastases, and a SLAP MCC cell line consistently develops regional lymph node metastases from orthotopic allografts, as well as liver and lung metastases following tail vein injection. However, the complex nature of this model requires extensive breeding, with only a small fraction of progeny carrying the required complement of genetically-modified alleles to produce tumors. This makes the SLAP model suboptimal for basic and translational studies on MCCs arising in the autochthonous setting.
METHODS
By incorporating different oncogenic drivers and making several modifications in transgene design, we have generated a panel of next-generation mouse models in an effort to simplify production of VP MCC in mice.
RESULTS
VP MCC-like tumors arise in next-generation mice carrying three genetically-engineered alleles instead of the six or seven in SLAP mice, greatly reducing the amount of time and effort required to produce tumor-bearing mice. Depending on breeding protocol, final crosses using these new mouse models should yield litters with 50% of mice capable of developing tumors.
CONCLUSIONS
These simplified autochthonous VP MCC mouse models should help accelerate the pace of MCC research while complementing our ongoing studies using SLAP mice and cell lines.

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MERKEL CELL POLYOMAVIRUS INFECTION AND PERSISTENCE MODELLED IN SKIN ORGANOIDS

Presenter: Nicole Fischer
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BACKGROUND-AIM

Merkel cell polyomavirus (MCPyV) is the cause of most cases of Merkel cell carcinoma (MCC). It is one of the few known human tumour viruses and, due to its direct role in the development of this skin cancer, it is an excellent model for viral tumourigenesis and tumourigenesis in general. Current models for studying infection, persistence, and pathogenesis are highly limited, particularly for chronic human viruses such as MCPyV, which are extremely well adapted to their host.

METHODS

In this study, we use an induced pluripotent stem cell (iPSC)-derived, hair-bearing skin organoid (SkO) system to demonstrate the efficient infection, progression, and spread of MCPyV

RESULTS

By combining technologies with single-cell and spatial resolution, we demonstrate that iPSC-derived SkOs can support viral infection and long-term persistence under conditions that closely resemble those in humans.

CONCLUSIONS

This infection model provides a robust platform for understanding virus-immune system interactions during infection, testing treatment strategies to control reactivation, and mapping the processes involved in tumour development.

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MANIPULATION OF CHROMATIN STATE BY THE VIRAL ONCOGENE HPV E7
Presenter: Amelie Fradet-Turcotte

A. Fradet-Turcotte 1
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BACKGROUND-AIM
Mammalian DNA is tightly packaged into nucleosomes, the basic subunits of chromatin, whose organization must be dynamically regulated to permit DNA accessibility—crucial for replication, repair, and transcription. Our previous work identified the human papillomavirus (HPV) E7 oncoprotein as an interactor of RNF168, an E3 ubiquitin ligase central to the cellular response to DNA double-strand breaks and replication stress. RNF168 ubiquitylates histone H2A on nucleosomes flanking DNA lesions, influencing the outcome of repair.
METHODS
While we have shown that RNF168 is essential for viral replication, the functional consequences of its interaction with E7 remain unclear. Using proximity labeling (BioID), we recently uncovered a novel RNF168-regulated interaction between E7 and the chromatin remodeler CHD8 (chromodomain helicase DNA-binding protein 8). CHD8 belongs to a family of ATP-dependent enzymes that fine-tune histone-DNA interactions and modulate nucleosome spacing.
RESULTS
Here, I will discuss our findings that RNF168-mediated chromatin ubiquitylation unexpectedly counteracts CHD8-driven chromatin compaction, and present evidence suggesting that E7 alters this interplay.
CONCLUSIONS
These results provide new insight into how oncogenic viruses may manipulate host chromatin dynamics to support viral replication and potentially promote genomic instability.

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MCPYV ALTO IS A TUMOR SUPPESSOR
Presenter: Denise Galloway

N.J. Salisbury 1
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BACKGROUND-AIM
MCPyV is an unusual polyomavirus; it is the only human PyV that encodes a protein that is wholly overprinted in the second exon of LT. To distinguish it from MT we have named it ALTO, alternate large T open reading frame. MCC cell lines express ST and truncated form of LT, but do not express ALTO.
METHODS
When ALTO is transduced into MCC cells, their proliferation is inhibited.
RESULTS
We have shown that ALTO acts like a TNF receptor, binding TRAF 2/3 and Sqstm 1 to activate both canonical and non-canonical NFkB signaling and down regulates expression of ST and LT-t. In a positive feedback loop, RelA induces the expression of ALTO. Using co-expression of RelA in cells with replicating MCPyV allowed us to map new transcripts that encode ALTO.
CONCLUSIONS
We propose that ALTO contributes to establishing latency of MCPyV.

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LIPID-FREE, THERMOSTABLE MRNA VACCINES
Presenter: Robert Garcea

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BACKGROUND-AIM
Lipid nanoparticle (LNP) formulations of mRNA vaccines have played a pivotal role in combatting SARS-CoV-2 infections and are expected to be a useful vaccine modality against other pathogens. However, the instability of mRNA-LNP vaccines requires their storage at frozen temperatures, and these vaccines may also pose distinct manufacturing challenges.
METHODS
We have prepared thermally stable, lipid-free mRNA vaccines by first spray-drying the mRNA within glassy polysaccharide microparticles, followed by atomic layer deposition (ALD) to encapsidate the microparticles within protective nanoscopic alumina shells that provide temporally controlled antigen release. Analogous to the previous use of such technology to prepare protein-ALD vaccines (e.g., rabies, HPV), the mRNA release profile can be modulated in a single administration by applying specific numbers of coating layers to give the desired prime-boost delivery schedule.
RESULTS
Using mRNAs encoding for ovalbumin and the modified HIV envelope trimer protein N332-GT2 as model antigens, these alumina-coated mRNA preparations elicited robust immune responses in mice compared to LNP-based mRNA vaccines and were stable when stored for weeks at temperatures up to 40 °C. In addition, HIV gp120-ALD vaccines gave equivalent antibody titers to similar LNP-vaccines that required SMNP adjuvants.
CONCLUSIONS
Thus, alumina-coated mRNA vaccines may overcome limitations of current mRNA vaccines without using LNPs or other lipid-based carriers.

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THE CELLULAR DEACETYLASE SIRT1 IN HPV-DRIVEN CANCER
Presenter: Marisa Gariglio

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BACKGROUND-AIM
High-risk HPV oncoproteins E6 and E7 drive carcinogenesis by promoting the degradation of p53 and pRb. We recently identified a novel mechanism by which HPV16 suppresses p53 activity through the deacetylase SIRT1 (Lo Cigno et al., 2023). Pharmacological inhibition of SIRT1 restores acetylated, transcriptionally active p53, inducing cell cycle arrest and reducing the viability of HPV-positive cells. Beyond p53 regulation, emerging evidence suggests that SIRT1 may also play a critical role in DNA replication control.
METHODS
Transcriptomic profiling was performed on NOK and NOKE6/E7 cells treated with the SIRT1-specific inhibitor EX527, focusing on cell cycle and senescence-related pathways. Functional assays included flow cytometry, EdU incorporation, and PI/Ki67 staining. DNA damage was assessed via ©H2AX immunostaining and alkaline comet assay. SIRT1 interactors were identified by mass spectrometry, and acetylome profiling is ongoing.
RESULTS
In HPV16+ cells, SIRT1 inhibition led to downregulation of G2/M checkpoint genes and upregulation of senescence-associated markers. Functionally, EX527 treatment induced G2/M arrest, reduced EdU incorporation and Ki67 expression, and irreversibly blocked cell cycle re-entry upon drug removal. Importantly, these permanently arrested cells showed a marked increase in cell size, consistent with mitotic failure, and progressed toward senescence, as confirmed by the SenMayo signature, ®-galactosidase staining, and focus formation assay. No DNA damage was detected. Proteomic analysis identified MCM4 and MCM7—key components of the replication licensing machinery—as novel SIRT1 interactors, reinforcing its role in S-phase regulation.
CONCLUSIONS
This study uncovers a dual role for SIRT1 in HPV16-positive cells: suppression of p53 activity and control of DNA replication. SIRT1 inhibition selectively impairs proliferation in HPV+ cells by inducing irreversible G2/M arrest and senescence. These findings highlight SIRT1 as a promising therapeutic target in HPV-associated cancers.

A PROSPECTIVE, SINGLE-ARM, MULTI-CENTER CLINICAL STUDY OF THE IMMUNOGENICITY OF NONAVALENT HPV VACCINE ADMINISTERED PRIOR TO RENAL TRANSPLANTATION IN ADULTS
Presenter Marc Goodman

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BACKGROUND-AIM
Vaccine efficacy data from clinical trials have demonstrated that 4vHPV and 9vHPV vaccines protect against HPV-related disease and that the protection is long-lasting However, little evidence exists on the real-world impact and effectiveness of HPV vaccines among non-HIV infected adult populations known to be at high risk for HPV-related disease.
METHODS
Candidates for kidney transplant were enrolled from five centers in the US. 9vHPV vaccine immune response was measured at 12-months post-transplantation among 18-49 year-old kidney transplant recipients who received 1-3 doses of the vaccine at least 30 days prior to transplantation. Serum samples were analyzed for anti-HPV IgG antibodies.
RESULTS
Of the 32 participants who had a kidney transplant, 20 had HPV labs available 12 months post-transplant. Seronconversion ranged from 45% (HPV52) to 90% (HPV 16, 31, 33).
CONCLUSIONS
Results of our Phase IIb trial suggest that kidney transplant recipients who receive 1 doses of the Gardasil® 9 HPV vaccine 30 days prior to transplantation have relatively high HPV vaccine-type-specific seroconversion rates at 12-months post-transplantation

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INITIATION OF A PHASE I TRIAL IN WOMEN OF RG1-VLP, A BROAD-SPECTRUM CHIMERIC CANDIDATE VACCINE FOR THE PREVENTION OF HPV-INDUCED NEOPLASIA
Presenter: Reinhard Kirnbauer

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BACKGROUND-AIM
We have initiated a first in women randomized controlled trial of RG1-VLP, an experimental vaccine designed to protect against a broad spectrum of mucosal HPV types beyond the types targeted by licensed multivalent vaccines. This vaccine candidate is composed of HPV16 virus-like particles (VLP) that display on their surface repetitively the RG1-peptide, a conserved 20 amino-acid cross-neutralization epitope of HPV16 L2 N-terminus, which is broadly conserved among different types, plus aluminum hydroxide adjuvant.
METHODS
Following a dose-escalation design of 8, 16, or 32 µg VLP, the primary endpoint will be safety. Serological studies will inform dose finding and analyze the induction of cross-reactive and cross-neutralizing antisera. In addition, neutralizing activity of immune sera will be correlated with protection against genital challenge with pseudovirions (PsV) in a mouse passive transfer model.
RESULTS
Human beta HPV types have been implicated, adjunct to the main carcinogen UV-light, in the development of keratinocytic skin cancers in EV- and immunosuppressed patients such as organ transplant recipients or people living with HIV. We aimed at developing a broad-spectrum ®HPV vaccine targeting the minor structural protein L2. To identify epitopes that give rise to cross-reactive antibodies, we employed an innovative immunization strategy and generated monoclonal antibodies that neutralized several of the 8 ®HPV types tested (5/8/20/24/38/76/92/96), and by passive transfer protected mice against ®HPV PsV challenge. Following epitope mapping, chimeric HPV16L1-based VLP were designed that present conserved beta L2 cross-neutralization epitopes on the VLP surface. In mouse active and passive immunizations, chimeric VLP induced cross-neutralization against several tested ®HPV types and conferred protection against HPV5 and HPV24 PsV challenge.
CONCLUSIONS
In conclusion, these novel chimeric VLP elicit broad immunity against ®HPV types implicated in skin cancer development.

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R-LOOPS REGULATE THE HPV LIFE CYCLE
Presenter: Laimonis Laimins

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BACKGROUND-AIM
The levels of R-loops are increased in HPV positive cells, and this is critical for viral replication as well as transcription. R-loops are trimeric nucleic acid structures consisting of a hybrid between RNA and its complementary DNA strand along with the displaced single strand DNA. These are stable structures that form at promoter as well as termination regions. R-loops play important roles in the normal regulation of transcription initiation and termination; however, failure to resolve aberrant R-loops leads to DNA break formation.
METHODS
We have shown that R-loop levels are increased by over 10-fold in HPV positive cells, and maintenance of these high levels is necessary for viral replication and transcription. Our studies identified one class of R-loops that are present at similar sites in HFKs and HPV positive cells but significantly increased in the latter. We also identified a second group of R-loops that form only in HPV positive cells and are present adjacent to genes in specific pathways such as the innate immune pathway. Importantly RNA-seq analysis of cells in which R-loop levels were reduced by overexpression of the R-loop specific RNAse, RNAse H1, demonstrated that these structures repressed the expression of genes in innate immune pathway.
RESULTS
The E6 viral oncoprotein was found to be responsible for inducing high levels of R-loops through its inhibition of p53. Furthermore, the increase in R-loop levels in HPV positive cells was found to be due to stabilization and correlated with low levels of m6A modified RNAs present in these structures. The reduction in m6A levels in HPV positive cells is due to the combined actions of E6 along with the R-loop resolving helicase SETX. Knockdown of SETX or inhibition of m6A blocked maintenance replication in undifferentiated cells as well as amplification upon differentiation.
CONCLUSIONS
These studies identify the R-loop /m6A pathway as a critical regulator of the HPV life cycle

ROLE OF ESTROGEN IN IMMUNE EVASION BY PAPILLOMAVIRUSES
Presenter: Paul Lambert

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BACKGROUND-AIM
Prior studies using HPV16 transgenic demonstrated a role of estrogen in driving cervical carcinogenesis in mice by a mechanism that is cell-nonautonomous. Estrogen also contributes to cervicovaginal
carcinogenesis in a mouse papillomavirus (MmuPV1) infection model. This effect was mediated by an ability of estrogen to drive persistent infection by MmuPV1 and associated with a systemic reduction in the levels of circulating immune cells (Wang, Spurgeon et al, 2023 PNAS, Proc Natl Acad Sci U S A. PMID: 36917668). This led us to ask if estrogen also influences the incidence of MmuPV1-induced athogenesis at a cutaneous site.
METHODS
Mice were infected in their ears with MmuPV1 and treated with exogenous estrogen. Wart incidence and size were monitored over time. Blood was analyzed by FACS to measure levels of circulating immune cells.
RESULTS
We found that exogenous estrogen increased the severity of disease in the skin epidermis and this again was associated with a reduction in levels of circulating immune cells. This was observed in both female and male mice.
CONCLUSIONS
These data indicate that estrogen increases susceptibility of mouse papillmavirus-induced disease in both mucosal and cutaneous tissues.

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UNDERSTANDING HOW NON-CODING RNA NETWORKS CONTRIBUTE TO OROPHARYNGEAL CANCER OUTCOME
Presenter: Andrew Macdonald

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BACKGROUND-AIM
The global incidence of HPV-associated oropharyngeal squamous cell carcinoma (OPSCC) is rising and likely to overtake cervical cancer as the most common HPV-driven malignancy. Most HPV-positive OPSCC are caused by HPV16.
Despite showing a more favourable prognosis compared to HPV-negative OPSCC, these cancers still receive the same therapeutic regime. Differences in the clinical outcomes necessitates a deeper understanding of their underlying molecular mechanisms. Our research aims to identify key interactions between HPV16 and host factors driving carcinogenesis to explain specific disease outcomes.
Long non-coding RNAs (lncRNAs) are a class of RNAs >200 nucleotides long. Increasing evidence has indicated that lncRNA play various important roles in carcinogenesis; however, the underlying mechanisms are often unknown. We set out to explore the lncRNA molecular signatures within patients as a means to unravel the specific biological pathways contributing to discrete patient prognoses, offering potential biomarkers for predicting tumour aggression and enabling more targeted therapies.
METHODS
Whole transcriptome sequencing was performed on HPV16-positive and HPV-negative oral cavity tumours, comparing each with healthy proximal tissue.
RESULTS
Differential expression analysis highlighted lncRNAs dysregulated between HPV-positive and negative cancers.
Through our analysis, FAM151B-DT, emerged as significantly upregulated in HPV-positive head cancers, with expression patterns reflected in HPV-positive HNSCC cell lines and upregulation tied to HPV oncoprotein expression. At the start of our studies FAM151B-DT was an uncharacterised lncRNA. We will present our biochemical characterisation of this lncRNA and describe how its expression contributes to differences in the cancer phenotype in OPSCC.
CONCLUSIONS
This work represents the first analysis of differentially expressed lncRNAs in HPV-positive versus HPV-negative HNSCC, based on internally sourced and sequenced patient data.

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VIRAL RECEPTOR DYNAMICS AND SIGNALING IN JC POLYOMAVIRUS ENTRY
Presenter: Melissa Maginnis

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1University of Maine

BACKGROUND-AIM
JC polyomavirus (JCPyV) infects the majority of the population causing a lifelong persistent infection in the kidney. In severely immunocompromised individuals, JCPyV can become reactivated in the central nervous system, infecting glial cells, astrocytes and oligodendrocytes, which are critical for myelin production. Viral infection and cytolytic destruction of glial cells leads to development of the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML), for which treatment options are limited. In order to identify targets for development of antiviral therapies, it is essential to define virus-host cell interactions that drive infection. Virus-receptor interactions and viral-induced signaling pathways are major regulators of tissue tropism and viral disease outcomes. JCPyV can utilize the serotonin 5-hydroxytryptamine (5-HT2) receptors to mediate internalization, and the virus can activate the mitogen-activated protein kinase pathway (MAPK); however, the mechanisms by which JCPyV utilizes these cellular factors to facilitate infection is not fully understood.
METHODS
The methods utilized include super-resolution Fluorescence Photoactivation Localization Microscopy (FPALM), viral infectivity assays, confocal microscopy, flow cytometry, high-throughput screening, receptor pull-down assays, and In-cell Western (ICW) assays.
RESULTS
We have identified the spatial arrangement and dynamics of the 5-HT2Rs during entry. Further, we have defined that 5-HT2 receptor-associated scaffolding proteins, including beta-arrestin, are recruited to the receptor to mediate viral internalization through a clathrin-mediated endocytosis pathway. Using a high-throughput drug screen we identified inhibitors that target cellular receptors and limit recruitment of beta-arrestin, resulting in reduced viral entry and JCPyV infection.
CONCLUSIONS
These findings highlight the importance of viral receptors and signaling pathways in JCPyV infection and illuminate potential targets for antiviral treatments.

REMODELING OF THE RIBOSOME QUALITY CONTROL BY VIRAL UBIQUITIN DECONJUGASE
Presenter: Maria G. Masucci

M. Masucci 1
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BACKGROUND-AIM
The translation of viral mRNAs is challenging due to the presence of long repetitive sequences, complex secondary structures, suboptimal codon usage, and the frequent occurrence of nucleotide misincorporations, which can slow down translation and trigger the Ribosome Quality Control (RQC) that regulates the fidelity of protein translation, ribosome recycling, and the activation of ribosomal and integrates stress responses.
METHODS
The strategies adopted by viruses to counteract the potential antiviral effects of the RQC are poorly understood. We found that the viral ubiquitin deconjugase (vDUB) encoded in the large tegument protein of the human pathogenic herpesviruses Epstein-Barr virus (EBV), Human cytomegalovirus (HCMV), and Kaposi sarcoma virus (KSHV) share the capacity to counteract the ubiquitination and UFMylation of ribosome subunits that are induced by ribosome stalling and collision.
RESULTS
The vDUB-mediated inhibition of the RQC and ER-RQC ligases promoted the readthrough of stall-inducing mRNA, rescued model substrates from proteasome- and lysosome-dependent degradation, and inhibited ER-phagy.
CONCLUSIONS
The EBV-encoded vDUB induces a GCN2-dependet integrated stress response, which enhances the translation of viral proteins and the release of infectious virus, pointing to a pivotal role of the vDUB in the translation reprogramming that enables efficient virus production and may prime the infected cells for malignant transformation.

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THE POLYMERASE MILIEU AND MECHANISMS OF HPV REPLICATION
Presenter: Kavi Mehta

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BACKGROUND-AIM
Cells experience tens of thousands of DNA lesions per day that must be repaired to ensure genetic stability. Without repair, mutagenesis, or genomic instability result. The replication stress response is important in responding to DNA damage and stress to prevent cancers. HPVs activate the human replication stress response, recruit host factors to the viral genome, and induce genomic instability.
METHODS
iPOND-MS/heliPOND-MS are quantitative methods for characterizing the host and viral replisome. Our studies, in patient cells and HPV positive neonatal foreskin derived cells that episomally maintain the HPV-31 genome, indicate a recruitment of a milieu of polymerases PolΣ, Pol™, Polκ, and translesion synthesis polymerases (TLS). Using a systematic approach, we are defining the polymerases that PVs use for replication through the differentiation-dependent lifecycle and how subversion of these polymerases influence mutagenesis in the host.
RESULTS
Our studies indicate that polymerases such as Polκ, Polz, and Polǀ are differentially recruited to HPV genomes both during viral maintenance and amplification. Literature recently described a potent and specific small-molecule inhibitor (CD437) of Polκ. Nanomolar concentrations of the inhibitor induces a robust replication stress response in HPV positive cells. Further, treatment of episomal cell lines influences both viral maintenance and viral amplification. Treatment of HPV-16 (-/+) primary matched patients demonstrate hypersensitivity to low-levels of Pola inhibitor in both novel live competition assays, organotypic raft cultures, and viability assays in HPV-positive cells indicating potential therapeutic potential.
CONCLUSIONS
Ongoing studies are querying the importance of other host polymerases and replicative intermediates across the HPV life cycle to define the core, minimal viral replisome.

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ONCOGENIC ENHANCER ARCHITECTURE AT SINGLE-CELL RESOLUTION IN KAPOSI SARCOMA TISSUE

Presenter: JJ Miranda

J. Miranda 1
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BACKGROUND-AIM

The Kaposi sarcoma-associated herpesvirus (KSHV) is an etiological agent of cancer. Since its discovery decades ago, the field has excelled at laboratory studies of determining how KSHV infection modulates host chromatin and transcription. Particular challenges remain, however, in studying actual tissue. Here we present our preliminary results studying hard-to-find clinical specimens with frontline genomics methods.

METHODS

We successfully processed a KS skin lesion for multiome single-cell ATAC-seq and single-cell RNA-seq experiments. This approach simultaneously measures chromatin accessibility and gene expression at single-cell resolution. Feature linkage identifies functional enhancers that regulate specific genes.

RESULTS

Our resulting map of chromatin interactions from actual cancer tissue represents a step beyond cell culture studies. Novel enhancers were identified compared to laboratory work studying a model of primary effusion lymphoma, another KSHV-associated malignancy. Moreover, we uncovered both positive and negative regulatory elements proximal to known oncogenes.

CONCLUSIONS

The overall impact is identification of potential modulators of KS. Elucidating regulatory architecture and circuitry could identify enhancers and transcription factors that may be essential for growth and also serve as potential targets in future treatments.

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VAGINAL MICROBIOME, METABOLOME AND CYTOKINE PROFILES IN WOMEN LIVING WITH AND WITHOUT HIV WITH CIN
Presenter: Anna-Barbara Moscicki

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BACKGROUND-AIM
Women living with HIV (WHIV) are more likely to have HPV persistence and cervical precancers/cancers and are also known to have vaginal microbial dysbiosis (VMD-defined as non-lactobacillus dominated [NLD] microbiome) which has recently been associated with HPV precancers in healthy women. VMD is a risk for acquiring HIV, hence its unclear whether VMD is a preexisting condition in WHIV or influenced by HIV. Little is known about HPV and the vaginal microbiome (VM) in women with perinatally-acquired HIV (WPHIV) which may inform us as to the relationships between HIV, VM and HPV.
METHODS
Two studies will be discussed. The first is a study of WPHIV where we found that VMD was the predominant VM suggesting that HIV directly affects the VM. There were colposcopic abnormalities associated with VMD but not the presence of CIN which may suggest VMD associated inflammation. Bacteria species associated with VMD, Prevotella bivia and Prevotella disiens, showed trends for an association with hrHPV. Metabolome patterns were also seen. Indole-3-lactate, which is involved in immune regulation and inflammation reduction, was associated with CIN 1 (vs no CIN). The second study examined women from a longitudinal study of HPV in healthy women who were HPV 16 infected and went onto develop CIN 2+ and were compared to women with HPV 16 who did not develop CIN 2 (negative control). The women who went onto CIN 2+ had two samples tested for microbiome, metabolome and cytokines (A:pre-CIN 2+ and B:at the CIN 2+ visit).
RESULTS
VMD was more likely to be present at B compared to negative control women. Visit A had a more mixed picture with a microbiome profile more similar to the controls. Of interest, there were individuals who at visit A had L. crispatus dominated VM (considered healthy) but went onto developing CIN 2+ strongly suggesting that the change in the VM (to non-Lactobacillus dominated) was the risk for the development of CIN 2+ and this was not associated with HPV 16 alone. Metabolite clusters were also noted to have increased expression at visit B compared to controls.
CONCLUSIONS
Cytokine expression was also enhanced at visit B compared to controls and visit A. These results will be discussed in the context of HPV clearance, development of CIN 2+ in those with and without HIV.

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PANHPVAX: A FIRST-IN-HUMAN PHASE I DOSE-ESCALATION TRIAL IN HEALTHY VOLUNTEERS OF A VACCINE TARGETING HUMAN PAPILLOMA L2 ANTIGEN
Presenter: Martin Müller

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BACKGROUND-AIM
Human papillomaviruses (HPV) are highly oncogenic viruses responsible for the majority of cervical cancers, as well as cancers of the vulva, vagina, anus, penis, and oropharynx. Existing vaccines protect against only about half of the relevant HPV serotypes, underscoring the need for broader protection against most oncogenic HPV strains to help reduce the incidence of these cancers.
METHODS
To address this, we are conducting a first-in-human, dose-escalating Phase I clinical trial of the PANHPVAX vaccine. This next-generation vaccine aims to provide broad protection against a wide range of oncogenic HPV serotypes, including those associated with genital warts.
RESULTS
Three cohorts have been initiated, with cohort #1 successfully completed, demonstrating promising results in terms of tolerability and immunogenicity. We are currently preparing a fourth cohort with a higher dose to determine the most effective dose and establish the recommended Phase II dose for the next stage of clinical development. An interim analysis of the preliminary safety and immunogenicity of cohorts 1–3 will be discussed.
CONCLUSIONS
The vaccine shows a good safety profile. Also, induction of HPV L2 and scaffold-specific immune responses could be detected.

NON-PROTEIN TARGETS AND EFFECTORS OF HPVS
Presenter: Karl Munger

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BACKGROUND-AIM
The HPV E6 and E7 proteins are the oncogenic drivers in HPV-associated cancers. They lack intrinsic enzymatic activity and promote carcinogenesis by targeting and subverting key cellular regulatory circuits. Many putative host protein targets and effectors of E6 and E7 have been identified. However, less than 5% of the human transcriptome encodes proteins, and the interactions of E6 and E7 with non-coding RNAs-particularly long non-coding RNAs (lncRNAs), remain largely unexplored.
METHODS
We previously reported that expression of HPV16 E6 and E7 leads to widespread dysregulation of host lncRNA expression. LncRNAs, defined as non-coding transcripts longer than 200 nucleotides, are known to interact with DNA, RNA, and proteins, and have been implicated in the regulation of diverse biological processes. Over the past several years, we have focused on elucidating the role of the DINO (Damage Induced Noncoding) lncRNA in the HPV life cycle and pathogenesis. Originally identified as a transcriptional target and positive regulator of the p53 tumor suppressor (PMID: 27668660), we subsequently demonstrated that ectopic expression of DINO can restore activity to the dormant p53 tumor suppressor in HPV-positive cervical cancer lines (PMID: 32546626).
RESULTS
Our recent studies suggest, however, that DINO functions beyond a simple p53-regulated p53 effector.
CONCLUSIONS
We are currently investigating DINO's role in the differentiation-dependent HPV life cycle, as well as its activities in cells lacking functional p53.

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ALTERNATIVE 3D CHROMATIN ARCHITECTURE IN VIRUSES LEADS TO PATHOGENESIS

Presenter: Donna Neumann
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BACKGROUND-AIM

In cellular genomes and in DNA viruses, CTCF insulators control transcription over long distances in a three-dimensional (3D) manner by nucleating and maintaining chromatin loops. In the context of human cells, chromatin loops are maintained to promote appropriate gene expression under normal conditions, while rearrangement or alterations to chromatin loops result in inappropriate gene expression that can lead to the onset of diseases, such as cancer. All three classes of human herpesviruses have been shown to utilize long-range chromatin interactions to maintain appropriate viral gene expression profiles during latent infections, and therefore, it seems likely that alterations to or rearrangements of 3D chromatin loop structures in latent herpesvirus genomes may be required to facilitate reactivation, and subsequent viral pathogenesis. To test this, we used well characterized models of HSV-1 latency and reactivation to determine how changes to latent genome 3D chromatin architecture affects pathogenesis and gene expression.

METHODS

We quantified the abundance of and the changes in long-range interactions using a circular chromosome conformation capture method known as UMI-4C-seq (Unique Molecular Identifier 4C-sequencing). UMI-4C-seq is a technique that provides a robust method of capturing and quantifying long-range interactions by incorporating unique molecular identifiers (UMIs) combined with specific viewpoint primers that assess one-to-all changes in long-range chromatin interactions. Interactions were mapped in two HSV-1 viral genomes (wt and genomes lacking a loop-nucleating CTCF insulator) both during latency and following reactivation.

RESULTS

Cis-interaction peaks across four different viewpoints were quantified. Viral genomes lacking the loop-nucleating insulator displayed altered chromatin organization compared to wt. Further, in genomes lacking the loop-nucleating insulator, there were losses to specific long-range cis interactions that mapped to genes that are required for efficient reactivation. Taken together, these results suggest that the 3D chromatin structure of the latent viral genome is important for the virus's ability maintain latency and to reactivate, causing dramatic changes in pathogenesis phenotypes.

CONCLUSIONS

Our results show that "normal" wt virus long-range chromatin interactions are required for the establishment of latency in HSV-1 and that these interactions are necessary for viral genomes to reactivate and cause pathogenesis. Altered chromatin loops that result from deletion of loop-nucleating insulators reshapes the viral chromatin landscape, leading to a more accessible and dynamic regulatory environment that influence HSV-1 transcriptional programs in both latent and reactivated states of the lifecycle, and can likely be applied to understanding chromatin dynamics of other DNA viruses and pathogenic states associated with those microbes.

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TRANSCRIPTIONAL REPROGRAMMING OF HOST GENE EXPRESSION BY ONCOGENIC HPV
Presenter: Joanna Parish

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BACKGROUND-AIM
Oncogenic HPVs are directly linked with approximately 20% of oropharyngeal malignancies globally which is trending upwards. However, there is a large disparity in prevalence between different HPV types at this anatomical site, with HPV16 accounting for >90 % of cases whilst HPV18 is rarely found (<3 %). The reason for this stark inequality in prevalence in currently unknown, but one theory is that HPV-type specific modulation of immune signalling dictates persistence and carcinogenic potential at particular anatomical sites. This is particularly importantforthe tonsil, given its role as a secondary lymphoid organ containing a plethora of immune cells in close proximity to the surrounding stratified squamous epithelium.
METHODS
In this study, we have developed primary, human tonsil keratinocyte cultures to investigate differential host transcriptional reprogramming by high-risk types HPV16 and HPV18. Multi-omic sequencing (ATAC- & RNA-Seq) was carried out following viral establishment. We hypothesised that there would be differential alterations in immune signalling pathways between HPV16 and HPV18 and we tested this theory using Gene Set Enrichment Analysis (GSEA).
RESULTS
This analysis revealed HPV16 significantly downregulated pathways involved with the innate immune response when compared to the HPV18-genome containing donor-matched cultures. We go on to show that key genes expressing immune signalling molecules and cytokines are repressed by HPV16 and that this repression is maintained in the presence of external stimuli. These data show that HPV16 can suppress key immune signalling pathways much more effectively than HPV18 in tonsil epithelia.
CONCLUSIONS
This transcriptional reprogramming of the host immune response may contribute to the significant disparity observed between HPV type prevalence in HPV-induced oropharyngeal malignancies.

THE DYNAMICS OF KSHV INFECTION IN THE CONTEXT OF PREVALENT CO-INFECTIONS IN SOUTH AFRICA

Presenter: Georgia Schäfer

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BACKGROUND-AIM
Despite the high prevalence of latent Kaposi's sarcoma-associated herpesvirus (KSHV) infections in patients from endemic areas with high human immunodeficiency virus (HIV) prevalence, KSHV lytic reactivation in the context of other co-infections is not well understood. While Kaposi's sarcoma is the most common AIDS-related malignancy world-wide, other KSHV-associated pathologies caused by lytic KSHV infection manifesting with severe inflammatory symptoms are often either underdiagnosed due to invasive diagnostical procedures or misdiagnosed due to overlapping clinical presentations mimicking other highly prevalent infectious diseases such as tuberculosis (TB) or, more recently, COVID-19.
METHODS
We conducted several cross-sectional clinical studies in South Africa assessing blood KSHV VL as a proxy for lytic reactivation in the recruited patient cohorts.
RESULTS
Our studies provide strong clinical evidence that blood KSHV VL was associated with mortality in critically ill HIVinfected patients with suspected TB or in severe COVID-19 patients, respectively. Moreover, we also found that high and repeated exposure to SARS-CoV-2 in unvaccinated non-hospitalised people living with HIV (PLWH) on anti-retroviral treatment (ART) led to reactivation of KSHV, which may have long-term consequences for tumorigenesis outlasting the pandemic. Intriguingly, while their HIV VL and CD_4 count responded to ART, selected patients displayed persistent and uncontrolled KSHV viremia correlating with an increase in inflammatory markers.
CONCLUSIONS
Cumulatively, these studies highlight the importance of assessing and monitoring KSHV viremia in HIV care, particularly in the context of unexplained inflammation, to improve diagnosis and treatment of KSHV-associated pathologies associated with lytic KSHV infection.

REGULATION AND EXECUTION OF HUMAN PAPILLOMAVIRUS UPTAKE BY ENDOCYTOSIS
Presenter: Mario Schelhaas

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BACKGROUND-AIM
Entry of Human papillomaviruses is unique in various different ways: (i) after the initial binding event, viruses undergo capsid modifications that prime them for successful entry; (ii) virions cross the plasma membrane in a seemingly stochastic manner by endocytosis with a protracted residence time on the cell surface; (iii) endocytosis is achieved by a unique and unexplored clathrin-independent mechanism; and (iv) intracellular trafficking and nucelar import involve retrograde trafficking to the Golgi apparatus and tethering to mitotic chromatin, respectively.
METHODS
Here, we addressed how virions are primed for entry and interact with their receptors for this, and we studied, how virus endocytosis is executed by cellular proteins that typically act at sites distinct from the plasma membrane. For this, we used a variety of virological, cell biological, biophysical and imaging techniques.
RESULTS
Our data supports a model in which virus particle breathing is arrested already upon engagement of the binding receptor heparan sulfate proteoglycans, where specific gycan modifications are key. We show that basically all known secondary receptor candidates can be compensated for and likely do not act as bona fide uptake receptors. Moreover, we implicate the actin nucleation promoting factor WASH as scission factor stimulating branched actin polymerization for vesicle generation during endocytosis.
CONCLUSIONS
The complex mechanism of entry is likely adapted to the cellular niche of infection, and while we start to understand mechanistic details on the different steps of entry, only an incomplete picture yet emerges on how these details explain the niche of infection.

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ATTEMPTS TO IDENTIFY PHARMACOLOGICALLY TARGETABLE STEPS IN THE LIFE CYCLE OF KAPOSI SARCOMA-ASSOCIATED HERPESVIRUS (KSHV)
Presenter: Thomas Schulz

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BACKGROUND-AIM
Kaposi Sarcoma-associated Herpesvirus (KSHV) causes three human malignancies, Kaposi Sarcoma (KS), Primary Effusion Lymphoma (PEL), Multicentric Castleman's Disease (MCD) and an inflammatory condition (KICS). Commonly used nucleoside inhibitors of the viral DNA polymerase inhibit KSHV replication in tissue culture but are ineffective against KSHV-associated neoplasms. We wanted to explore the druggability of new points in the viral life cycle.
METHODS
KSHV replication assays, biochemical assays, structure biology, medicinal chemistry
RESULTS
Based on the molecular structure of the DNA-binding domain of the major KSHV latency protein LANA, we developed a small molecule that inhibits the binding of LANA to the viral latent origin of replication and latent viral replication. We explored the role of the KSHV K15 non-structural membrane protein in the KSHV life cycle. KSHV pK15 is one of the earliest viral proteins to be expressed after infecting lymphatic endothelial cells (LECs) with KSHV, is expressed in KS biopsies and recruits phospholipase C gamma 1 (PLCg1) to activate a number of intracellular signalling pathways and the expression of cellular angiogenic and inflammatory genes. We showed that pK15 facilitates the phosphorylation of PLCg1 by cellular Src kinases and obtained the molecular structure of a complex of the PLCg1 C-terminal SH2 domain with a pK15 peptide. We generated KSHV mutants with a pK15 that is deficient in recruiting PLCg1 and showed that recruitment of PLCg1 strongly facilitates viral replication during the first hours after infecting LECs. We devised short peptides that inhibit the pK15-PLCg1 interaction and pK15-dependent enhancement of PLCg1 phosphorylation. One small molecule inhibitor to emerge from this approach potently inhibited KSHV lytic replication but turned out to act via a different mechanism.
CONCLUSIONS
We have developed one inhibitor targeting the latent phase of KSHV replication and one potent inhibitor targeting the lytic phase by interfering with a cellular target.

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BIOMARKERS OF HEAD AND NECK CANCER IN LATIN AMERICA AND EUROPE
Presenter: Laura Sichero

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BACKGROUND-AIM
HPV-driven oropharyngeal (OPC) and oral cavity (OCC) squamous cell carcinomas prevalence varies worldwide. Our aim was to describe HPV-driven prevalence in several Latin American countries and Italy and explore the association with sociodemographic factors.

METHODS
We evaluated HPV DNA presence in OPC and OCC from Italy, Argentina, Peru, and three Institutions in Brazil, included in the HEADLAcE Consortium. HPV was genotyped by PCR-reverse line hybridization. In a subset of cases, we evaluated p16INK4a and HPV E6*I mRNA levels. Crude and standardized HPV-driven prevalences and 95% confidence intervals were estimated. The association between HPV-status and overall mortality was estimated using adjusted Cox proportional hazards models for OPC patients from Italy and two Brazilian Institutions.
RESULTS
912 patients (59.1% OCC and 20.9% OPC) were included. Prevalence of HPV-driven OPC was 24.7% (95% CI: 20.3-29.1) when defined by HPV DNA and p16 positivity, and 20.4% (95% CI: 16.2-24.6) when determined by HPV DNA and mRNA positivity. Crude prevalence ranged from <5% in Peru and the Brazilian HH center to 40-50% in Argentina, Italy and the Brazilian ACCCC center. After adjusting for possible confounders, the standardized prevalence was highest in Italy (IEO center). No significant differences were observed by sex, age group, race, or ethnicity. HPV prevalence was higher in never smokers and never drinkers. Regarding OCC, prevalences were much lower than in OPC (3.3% [95% CI: 1.8-4.9] based on concomitant HPV DNA and p16 overexpression, and 1.9% [95% CI: 0.7-3.1] based on HPV DNA and mRNA). Among OPC patients with follow-up data (N=265), after a median of 1.6 years, the adjusted risk of overall mortality was 69% lower in patients with HPV+ than HPV- tumours.
CONCLUSIONS
Our results support a role for HPV in a subset of OPC, and highlight the heterogeneity observed in samples from different geographic regions.

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EPITHELIAL STEMNESS AND INFLAMMATION ARE INTIMATELY LINKED IN HPV-DRIVEN CARCINOGENESIS
Presenter: Sigrun Smola

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BACKGROUND-AIM
Approximately 5% of all human cancers are caused by human papillomavirus (HPV) infection. Although the HPV oncoproteins and their transforming potential are well characterized, the process of malignant progression in vivo is not yet fully understood.
METHODS
Using immunohistochemical in situ analysis, 3D models as well as immunological and molecular analysis in vitro, we have highlighted the importance of the microenvironment and chronic inflammation for carcinogenesis.
RESULTS
We now provide evidence that inflammation is clinically and mechanistically linked to epithelial stemness in various entities of HPV-driven carcinogenesis.
CONCLUSIONS
Results from our study offer novel targets for intervention.

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CELLULAR PLASTICITY IN CANCER
Presenter: Katerina Strati

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BACKGROUND-AIM
Cellular plasticity and the process of epithelial to mesenchymal transition, underlies key changes in cell states during carcinogenesis and is emerging as a critical factor in the recurrence of disease and resistance to cancer therapy . Reactivation or deregulation of embryonic pathways is frequently linked to these processes. Using HPV-driven cancers as a model, I will discuss recent findings which suggest that cancer cells co-opt pathways fundamental during embyogenesis. I will present past and ongoing work which explores the regulation and role of Oct4 in cancers and somatic cells.
METHODS
We have used overexpression, knockdown and knockout strategies in immortalized and cancer cells.
RESULTS
Evidence suggests that loss of Oct4 in immortalized and cancer cells consistently caused a lag in the G2M phase of the cell cycle, prompting us to investigate the accumulation of DNA damage. Using COMET assays, and by verifying phorphorylated gammaH2Ax accumulation we corroborated the increased levels of DNA damage in the cells. Remarkably, re-expression of Oct4 in these knockout cells rescues the observed phenotypes and mitigates DDR activation.
CONCLUSIONS
These results may have important implications regarding the role of Oct4 in preserving genomic integrity in stem cells as well as altering the response to DNA damage in cancer cells.

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EXPLORING THE MOLECULAR LANDSCAPE OF VULVAR SQUAMOUS CELL CARCINOMA
Presenter: Maria Lina Tornesello

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BACKGROUND-AIM
Vulvar squamous cell carcinoma (SCC) primarily arises through two distinct pathways: one associated with high-risk human papillomavirus (HPV) and the other linked to virus-independent lichen sclerosus-related carcinogenesis. Therapeutic strategies for vulvar SCC are not tailored to tumour subtypes, and more studies are needed to define the unique molecular characteristics of HPV-related and -unrelated types for more effective treatments. The aim of the study was to characterize the molecular landscape of HPV-associated and HPV-independent tumours.
METHODS
We investigated the presence of HPV and mutations in TERT promoter (TERTp) region by end-point PCR and droplet digital PCR (ddPCR), respectively. Transcriptomic analysis (RNA-seq) was utilized to identify missense mutations, upregulated genes and the activation of specific pathways associated to HPV and/or TERTp status of vulvar SCC. SiRNA mediated inhibition of telomerase was performed in vulvar cancer cell line SW954 carrying TERTp C228T to assess its effect on the expression of p53-related genes and cell proliferation.
RESULTS
HPV DNA was found in 37% of vulvar SCC with HPV16 as the predominant genotype (78% of all infections). Activating mutations in TERTp were identified in 45% of vulvar SCC and more frequently in HPV negative cases (52%). Telomerase mRNA expression was 2-fold higher in TERTp mutant versus not-mutant vulvar SCC. RNA sequencing showed different gene expression patterns between HPV-positive versus HPV-negative as well as between TERTp-mutant versus TERT-wild-type tumours. Silencing of the telomerase by siRNA resulted in the downregulation of some genes involved in cell cycle regulation.
CONCLUSIONS
In conclusion, vulvar SCC is a heterogeneous disease characterized by diverse molecular features associated with HPV infection and/or to telomeres activation. A comprehensive understanding of HPV status, the mutational repertoire, and the activation of oncogenic pathways will certainly contribute to more effective and personalized cancer therapies.

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REGULATION OF HPV ALTERNATIVE SPLICING BY PRMT1
Presenter: Koenraad M. Van Doorslaer

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BACKGROUND-AIM
Human papillomaviruses (HPVs) infect mitotically active basal keratinocytes. While most infections are immunologically cleared, a small subset of HPVs establish long-term, persistent infections resulting in oncogenesis. Balanced expression of early viral genes, specifically the oncogenes E6 and E7, are required to create a cellular environment permissive for viral replication. A balance between these oncogenes is strictly regulated through alternative splicing of polycistronic mRNAs, contributing to viral establishment and long-term persistence. Oncogenic HPV types regulate relative oncogene expression through RNA splicing and polyadenylation. How HPV regulates alternative splicing during early infection is an active research area. We previously identified protein arginine N-methyltransferase 1 (PRMT1) as a host factor upregulated by HPV18 infection in primary human cells. PRMT1 is the primary enzyme driving asymmetric arginine dimethylation of proteins, regulating various cellular processes critical to the HPV lifecycle
METHODS
To gain further insights into how HPV regulates PRMT1 activity, we used immunofluorescence to investigate the sub-cellular localization of PRMT1. We used methylated RNA-immunoprecipitation (MeRIP) and Nanopore direct sequencing to demonstrate that HPV18 mRNA is m6A modified. To determine methylation sites regulated by PPRMT1, we mutated consensus m6A sites and quantified alternative splicing.
RESULTS
We demonstrate that HPV relocates a portion of the cellular PRMT1 from the nucleus to the cytoplasm, and this translocation is important for PRMT1's ability to regulate viral splicing. PRMT1 inhibition was associated with increased viral m6A deposition, specifically on viral introns. Furthermore, we identify consensus m6A sites that are involved in the regulation of E6 splicing.
CONCLUSIONS
We will discuss a critical role of PRMT1 and viral m6A modifications in regulating alternative splicing, balanced oncogene expression, and, ultimately, the establishment of long-term infections.

DYNAMIC PROTEIN INTERACTIONS DURING ADENOVIRUS INFECTION
Presenter: Matthew D. Weitzman

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BACKGROUND-AIM
During the late stage of adenovirus infection, newly synthesized viral DNA genomes are packaged within a proteinaceous icosahedral capsid shell. Successful packaging and incorporation of all essential viral structural proteins leads to particle maturation. It remains unclear how capsid assembly and genome packaging are coordinated in the crowded nuclear environment to produce infectious progeny. Although not a component of completed infectious particles, the viral 52-55 kilodalton protein (L1-52K) co-purifies with immature particles and is essential for assembly of packaged particles, suggesting that L1-52K acts as a link between capsid assembly and genome packaging.
METHODS
We utilized classical techniques in molecular virology in combination with advanced microscopy, in vitro biochemistry, and nanoparticle tracking to investigate the role of the viral L1-52K protein in coordinated assembly of packaged viral particles.
RESULTS
We found that phase-separation of L1-52K is critical to formation of nuclear biomolecular condensates (nuclear bodies) that organize viral capsid proteins. Phase-separation of L1-52K is dependent on the sequence of its N-terminal intrinsically disordered region (IDR). Using temperature-sensitive and IDR mutants, we show that phase-separation of L1-52K and the localization of key components to viral condensates is a pre-requisite for assembly of packaged particles. In addition, we present evidence that the interaction of L1-52K with viral genomes may provide the stimulus that initiates assembly of packaged progeny particles. We further show that phosphorylation within the N-terminal intrinsically disordered region of L1-52K modulates viral condensates in vitro and in cells, promoting liquid-like properties over condensate hardening. We have developed ways to arrest the dynamics of adenoviral L1-52K condensates, which inhibited partitioning of capsid proteins into condensates and suppressed viral particle assembly.
CONCLUSIONS
Collectively, our data indicate that the assembly of packaged progeny particles is a highly coordinated process that requires the formation of viral biomolecular condensates. Our data support the concurrent model of adenovirus assembly and packaging. These findings suggest that approaches which target regulation of viral biomolecular condensates could have antiviral applications.

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YAP ACTIVATION DRIVES HPV CARCINOGENESIS
Presenter: Elizabeth A. White

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BACKGROUND-AIM
HPV E7 proteins bind and degrade the tumor suppressor PTPN14, a suppressor of the YAP1 oncoprotein. YAP1 is a transcriptional coactivator that drives epithelial cell self-renewal and is controlled by the multi-component Hippo signaling pathway. Research in our laboratory is on the mechanisms and consequences of PTPN14 degradation and YAP1 activation by HPV E7 proteins.
METHODS
We use engineered human keratinocytes to determine how HPV oncoproteins control YAP1 and related signaling pathways. We grow cells that express HPV oncoproteins, or in which the expression of PTPN14 or one or more components of the Hippo pathway is modified, in two- and three-dimensional tissue culture models.
RESULTS
To elucidate the mechanism by which PTPN14 degradation activates YAP1, we tested how HPV E7 alters signaling in the Hippo pathway. MST1/2 and LATS1/2 are core Hippo kinases. Active LATS1 kinase is phosphorylated on threonine 1079 and inhibits YAP1 by phosphorylating it on amino acids including serine 127. PTPN14 knockout or PTPN14 degradation by HPV18 E7 decreased phosphorylation of LATS1 T1079 and YAP1 S127.
To determine whether PTPN14 degradation and YAP1 activation are required for the carcinogenic activity of high-risk HPV oncoproteins, we used assays of primary keratinocyte lifespan extension. We found that high-risk HPV E7 proteins can extend keratinocyte lifespan only when they can bind and degrade PTPN14. Mutants of high-risk HPV E7 that are impaired in either RB1 inactivation or PTPN14 degradation individually lack the ability to extend keratinocyte lifespan but could complement each other in the same assay.
CONCLUSIONS
Experimental evidence and analyses of cancer genomic data support that YAP1 activity is an essential contributor to HPV-mediated carcinogenesis and to the ability of HPV-infected cells to persist in dynamic epithelial tissues. Our long-term goal is to solidify the role of PTPN14 and YAP1 in HPV biology and disease.

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METABOLIC DEREGULATION BY ALPHA AND BETA HPV TYPES AND IMPACT ON CELLULAR PROLIFERATION
Presenter: Katia Zanier

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BACKGROUND-AIM
The functions of tumor suppressor p53 are impaired by high-risk HPVs (hr-HPVs) from the alpha and beta genera via different mechanisms. While hr-‹HPVs target p53 to degradation through formation of an E6/E6AP/p53 complex, the hr-HPV38 virus from the ß2 group induces stabilization of p53 levels but, at the same time, promotes the expression of the □Np73‹ oncogenic isoform, an antagonist of p53/p73 transcription.
It is well established that p53 controls the expression of genes coding for metabolic regulators. One of these genes is ABCA1, which codes for a cholesterol transporter and sensor of cholesterol intracellular trafficking and which acts to inhibit the maturation of Sterol Regulatory Element Binding Protein (SREBP) transcription factors.
METHODS
Here, we performed gene expression and cellular analyses.
RESULTS
We found that ABCA1 expression is strongly repressed in human keratinocytes transformed by the E6/E7 oncoproteins of either ‹-HPV16 or ß-HPV38 as compared to primary cells, leading to constitutive activation of the mevalonate pathway. This, in turn, activates YAP-signaling and, consequently, the transcription of pro-proliferative gene targets. Treatment with cerivastatin induces cell death specifically in E6/E7-transformed keratinocytes.
CONCLUSIONS
These results suggest a potential benefit of using anti-cholesterol drugs for the treatment of hr-HPV lesions.

LARGE-SCALE DNA ANALYSES REVEAL COMPLEX, VARIABLE STRUCTURES RESULTING FROM REARRANGEMENTS OF HPV AND HUMAN DNAS IN AN HPV-POSITIVE SQUAMOUS CARCINOMA CELL LINE

Presenter: Eleanor Agosta

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BACKGROUND-AIM
Human papillomaviruses (HPV) cause most cervical carcinomas and substantial fractions of oropharyngeal, anal, vulvar, penile, and vaginal carcinomas. HPV DNA replicates as extra-chromosomal episomes during infection and integrates into the human genome in nearly all cancer cases. In tumors, it can exist as extra-chromosomal DNA (ecDNA) circles and/or as chimeric heterocatemer tandem repeats covalently linked with human DNA. The presence of ecDNA is associated with poor patient prognosis across cancer types. However, distinguishing whether particular amplified DNAs in tumors are ecDNA and/or intrachromosomal tandem repeats (such as heterocatemers) presents a technical challenge.
METHODS
Therefore, to accurately characterize HPV integration structures, we applied a battery of DNA analysis techniques, including Nanopore long-range sequencing, Bionano optical genome mapping (OGM), and single-cell fluorescence in situ hybridization (FISH), to the HPV16-positive human oropharyngeal squamous cell carcinoma cell line UM-SCC-47.
RESULTS
We precisely defined the structure of the integrated HPV16 DNA, determined the human-virus DNA junctions, and mapped a repeated, 23 kbp heterocatemer at single-base-pair resolution. OGM revealed that the 23 kbp heterocatemer occurred in tandem arrays of various lengths ranging from a single unit to greater than 27 tandem units. These large-scale arrays (up to 700 kbp) were further rearranged with adjacent human DNA in even larger-scale structures. Additionally, FISH analysis revealed that every cell had intrachromosomal HPV16 associated with human DNA, with a subset of cells also containing ecDNA.

THE IMPACT OF EBNA2 AND LMP1 ON T CELL DYNAMICS IN PEDIATRIC EBV INFECTION: A STUDY IN ARGENTINE TONSILS

Presenter: Maria Eugenia Amarillo

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BACKGROUND-AIM

EBV enters through the tonsil, where the host immune system plays a key role in control primary and persistent infection. This study aims to characterize T cell subsets in the tonsils of EBV-infected children in Argentina.

METHODS

We studied 32 patients undergoing tonsillectomy. Immunohistochemistry (IHC) was performed to detect LMP1 and EBNA2 viral proteins, expressed as +cells/cm2 in histological regions: germinal center (GC), mantle (M), interfollicular (IF) and subepithelial (SE). EBERs was detected by in situ hybridization (ISH). EBV infection status was defined by serology. Fresh tonsils were analyzed by multiparametric flow cytometry to assess T cell subsets.

RESULTS

LMP1 expression was predominantly expressed in the IF region, while EBNA2 was similarly expressed in GC and IF regions. No significant differences were observed in CD4+ follicular helper T-cell (Tfh) subpopulations across EBV infection status or latency groups ($p>0.05$). EBNA2 total (T) cell count positively correlated with Tfh ($p=0.047$, $R=0.3928$) and with Tfh in IF and M regions ($p=0.020$, $R=0.453$; $p=0.035$, $R=0.4152$). LMP1 showed only a positive correlation with Tfh subsets at the SE region ($p=0.002$, $R=-0.573$). Th1, Th2, and Th17 frequencies did not differ by infection status ($p>0.05$), but Th1 levels tended to be higher in latency III cases ($p=0.0583$). Th1 also correlated with EBNA2 T count ($p=0.0218$, $R=0.4861$).

Latency III patients exhibited lower CD8+ T cells ($p=0.042$), with a negative correlation between CD8+ T cells and EBNA2 T count ($p=0.044$, $R=-0.405$).

CONCLUSIONS
In children from Argentina, EBNA2 could be associated with an increase in Tfh and Th1 cells probably recruited to control the expression of this oncogenic latent viral protein, along with a reduction of CD8+ T cells. In Argentina, with a high prevalence of pediatric EBV-associated lymphomas, these scenario in tonsils may offer insights into early lymphomagenesis.

E6-ENCODED BY CANCER-CAUSING HPV INTERACTS WITH CYCLIN-DEPENDENT KINASE 5 (CDK5).
Presenter: Siaw Shi Boon

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BACKGROUND-AIM
Human papillomavirus (HPV) E6 oncoproteins interact with various host proteins and perturb their functions. We previously found that E6-encoded by cancer-causing HPV genotypes interact with several kinases, including Aurora kinases and polo-like kinases. We recently found that cyclin-dependent kinase 5 (CDK5) is a previously unidentified host target. This study delineates the mechanism of E6-CDK5 interaction and whether this host-protein complex poses therapeutic value.
METHODS
We performed a series of in vitro binding assays to verify the E6-CDK5 complex formation. Leveraging virtual screening and molecular docking, followed by in vitro screening and cell-based assays, we identified compounds that can inhibit E6-CDK5 interaction.
RESULTS
E6 preferentially interacted with nuclear CDK5. This interaction stabilized E6. From the virtual and in vitro screening, we identified a compound, CUHK- HPVE6CDKi-CP005 disrupted E6-CDK6 and E6-E6AP association, conferring to rescued p53. We also observed that CUHK-HPVE6CDKi-CP005 can dampen cancer phenotypes driven by HPV, including cell proliferation, invasion, migration, and transformation.
CONCLUSIONS
The findings from this study provide fundamental molecular insight into the oncogenic link between CDK5 and E6. More importantly, CUHK-HPVE6CDKi-CP005 demonstrated a high selectivity and inhibitory effect on HPV-positive cancer cells, underlining the therapeutic value of this compound.

MOLECULAR CHARACTERIZATION OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) LYTIC REACTIVATION IN THE CONTEXT OF COVID-19
Presenter: Katrin Bratl

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BACKGROUND-AIM
Kaposi's Sarcoma-associated Herpes Virus (KSHV), an oncogenic gamma-herpesvirus, is highly prevalent in Sub-Saharan Africa (SSA) and exhibits a biphasic (latent/lytic) life cycle. Reactivation of KSHV can be triggered by environmental factors, co-infections, and inflammation. This study assessed the mechanisms of KSHV lytic reactivation in the context of COVID-19, which in SSA occurred against a backdrop of highly prevalent infectious diseases. Our previous clinical studies suggested a link between lytic KSHV infection and adverse COVID-19 outcome; however, cause–effect relationships could not be determined due to the cross-sectional study designs.
METHODS
To support these clinical findings on a molecular level, an in vitro model was established to investigate if co-infection with SARS-CoV-2 and/or the COVID-19 associated "cytokine storm" may induce lytic reactivation of KSHV. We successfully established three different rKSHV-latently infected cell lines which could be lytically reactivated using the known chemical stimulant, Sodium Butyrate (NaB), namely HuARLT (human endothelial cells), BJAB (human B cells) and VeroE6 (Green monkey kidney epithelial cells).
RESULTS
We demonstrated KSHV lytic reactivation by measuring newly produced virions in the cell culture supernatant upon infection with SARS-CoV-2 pseudovirions (PsVs), but not VSV-G control PsVs, and with a SARS-CoV-2 clinical strain. In addition, we observed KSHV lytic reactivation in the context of a "cytokine storm" using a direct cell-to-cell co-culture model with stimulated THP-1 human macrophages.
CONCLUSIONS
Our data suggest that SARS-CoV-2 infection can trigger lytic KSHV reactivation by both direct (viral infection) and indirect (associated "cytokine storm") mechanisms. These observations are currently further characterised using MS-based proteomics to identify key player proteins that can be potentially targeted to inhibit lytic reactivation.

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REGULATION OF THE EXPRESSION OF MATRIX METALLOPROTEINASES INHIBITORS BY MIRNAS IN CELLS EXPRESSING HPV GENES
Presenter: Amanda Campos Nocera

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BACKGROUND-AIM
The extracellular matrix plays a vital role in tissue homeostasis by providing mechanical and biochemical support to cells. This is ensured by the balance between the deposition, and degradation of its components. The latter function is primarily conducted by enzymes such as matrix metalloproteinases, whose functions are downregulated by the reversion-inducing cysteine-rich protein with Kazal motifs (RECK) and tissue inhibitors of metalloproteinases (TIMPs). The dysregulation in the levels and activities of these proteins has been associated with the progression of cancer.
Our group has observed lower levels of RECK in samples from cervical cancers, as well as a negative correlation between the expression of the HPV16 oncogenes E6/E7, and RECK-TIMP-2 in keratinocytes. However, the mechanisms involved in this process have not yet been elucidated in HPV associated pathologies.
Therefore, this study aims to investigate the players and the potential influence of HPV in these mechanisms. We hypothesize that miRNAs contribute to them.
METHODS
In silico analyses were performed to predict a miRNA that could regulate RECK and TIMP-2 using the TargetScan, microT-CDS and miRDB tools. Subsequently, this miRNA was inhibited using antisense oligonucleotides in HeLa cells. RECK and TIMP-2 levels were assessed by RT-qPCR and Western Blot. The viability of these cells after the inhibition was examined by colorimetric assays and cell counting.
RESULTS
miR-21 was selected through in silico analyses. When the miRNA is inhibited, the levels of RECK and TIMP-2 proteins are increased. Additionally, there are higher levels of p53 and the opposite is observed for E6AP, along with a lower number of viable cells and a higher number of dead cells upon inhibition.
CONCLUSIONS
These results indicate that miR-21 is a potential regulator of RECK and TIMP-2 in HPV-positive cell lines. Furthermore, this molecule also appear to have significant implications regarding the proliferative potential and death mechanisms of these cells.

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PELP1 MEDIATES HUMAN PAPILLOMAVIRUS TYPE 16 E6*I ISOFORM PRODUCTION IN CERVICAL CANCER
Presenter: Andrea Cerasuolo

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BACKGROUND-AIM
Persistent infection with HPV and constitutive expression of E6 and E7 oncogenes are the main cause of cervical squamous cell carcinoma (CSCC). The differential production of E6, E6*I and E7 is modulated by several RNA-binding proteins during cervical carcinogenesis. The PELP1 associates with PRMT6 and SRSF2, and participates in alternative splicing processes. In this study, we investigated the role of PELP1 in HPV16 E6*I production in CSCC.
METHODS
The expression of PELP1, PRMT6 and SRSF2 as well as of HPV16 E6 and E6*I was analysed in 14 CSCC and in 25 cervical intraepitehlial neoplasia (CIN) by RT-qPCR. SiHa, HeLa, HT-3, NTERA-2, PCA-5 and PCA-23 cell lines were transduced with LXSN retrovirus carrying HPV16 E6 or E6E7 ORFs and analyzed for PELP1, PRMT6, SRSF2 and E6, E6*I expression by RT-qPCR. The E6 and E6*I levels were validated by ddPCR. PELP1 expression was silenced in SiHa cells by siRNA transfection and protein levels were analysed by western blot.
RESULTS
PELP1 was found overexpressed in HPV-positive CSCC compared to low-risk HPV-positive CIN ($p<0.01$), while SRSF2 was more expressed in low-risk HPV-positive CIN compared to CSCC ($p<0.01$). The E6*I/E6 expression ratio was higher in CSCC compared to CIN. PRMT6 expression showed no statistically significant difference between sample groups. Transduction of cell lines with LXSN E6 caused a 2 to 8 fold up-regulation of PELP1 ($p<0.05$), which was observed also at protein level. Moreover, PELP1 levels were shown to correlate with E6*I levels in all cell lines ($R=0.88$, $p<0.05$). PELP1 knockdown confirmed its role in E6 mRNA splicing.
CONCLUSIONS
HPV16 E6 up-regulates PELP1, which in turn enhances the E6*I isoform production in CSCC. These data suggest that PELP1 may have an important role in cervical carcinogenesis.
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EPIGENETIC REGULATION OF RECK PROMOTER ACTIVITY IN HPV ASSOCIATED TUMORS
Presenter: Fernanda Costa

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BACKGROUND-AIM
High-risk oncogenic HPV (HR-HPV) infection is associated with approximately 5% of all cancers, including cervical cancer and oropharyngeal squamous cell carcinoma (OPSCC). E6 and E7 oncoproteins can induce alterations in the expression and activity of extracellular matrix components, including matrix metalloproteinases and their regulators, such as the glycoprotein RECK (REversion inducing Cysteine rich protein with Kazal motifs). Previous studies from our group have linked the reduction of RECK levels to the expression of E6 and E7 oncogenes. Additionally, we observed downregulation of RECK in cervical cancer and precursor lesions. Mutations in the RECK gene are rare in cancers, suggesting the involvement of other molecular mechanisms for its silencing in HPV-positive tumors.
Here, we investigated the role of promoter methylation in the regulation of RECK expression in cells expressing HPV16 or HPV18 oncogenes. Finally, we analyzed the methylation status of CpGs islands located in the proximal RECK promoter region of HPV+ and HPV- OPSCC samples.
METHODS
The methylation status of the minimal RECK promoter was determined by pyrosequencing. This region was analyzed in primary human keratinocytes transduced with retroviral vectors carrying HPV11, HPV16, and HPV18 E6 and E7 sequences and in cervical cancer-derived cell lines. Besides, we analyzed 120 tumor samples from an OPSCC cohort of patients at the Brazilian National Cancer Institute.
SiHa and SW756 cells were treated with 30 μM 5-azacytidine for 72 hours. Total RNA was extracted, reverse transcription performed, and qRT-PCR carried out.
RESULTS
The 5-azacytidine assay results indicated the induction of RECK mRNA expression in the HPV+ cell lines SiHa and SW756. In addition, we observed that the expression of HR-HPV oncogenes was associated with differential methylation of CpG sites located in the minimal RECK promoter.
CONCLUSIONS
RECK promoter methylation may play a role in the down-regulation of RECK in the context of HPV-mediated carcinogenesis.

A BIOLOGICAL ACTIVE CELL PENETRATING PEPTIDE INHIBITING THE GROWTH OF HPV16-POSITIVE CERVICAL CANCER CELLS

Presenter: Luisa Dassi

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BACKGROUND-AIM
Cell penetrating peptides (CPPs) are positive charged moieties able to enter cell membranes and to deliver therapeutic cargos. Small peptides disrupting the HPV E6/E6AP complex, causing p53 degradation in HPV-related tumors, have been previously identified in a randomized peptide expression library. Such 15-mer peptide, named pep11, was shown to bind E6 and to abrogate p53 degradation selectively in HPV16-positive cancer cells. The aim of our study was to develop a modified pep11 carriyng a CPP amino acid sequence (CPP-pep11) and to evaluate its ability to penetrate and to inhibit the cell growth.
METHODS
Despite the difficulty of synthesizing the hydrophobic pep11, CPP-pep11 was successfully synthesized by using the Boc chemistry. The short CPP was added at the N-terminus end of pep11, facilitating the solubility of CPP-pep11. The HPV-positive cervical carcinoma cells SiHa were incubated with serial dilutions (0.5, 1, 2, 10 ⌈M) of biotin-labelled CPPpep11 (bio-CPP-pep11) for different time points (24, 48, 72 hours) and cell uptake as well as intracellular distribution was detected by confocal microscopy and by differential fractionation of cells and western blot.
RESULTS
The bio-CPP-pep11 was found to enter the cells without interfering with cellular viability and membrane integrity even at longer times of treatment. In addition, it was shown to localize into the cytoplasm and perinuclear area. Peptide cellular uptake was found directly correlated to its concentration. Limited peptide degradation was observed over time as demonstrated by bio-CPP-pep11 detection in microscopy and western blotting analyses also at extended times. The evaluation of peptide internalization mechanisms and restoration of p53 protein levels is ongoing.
CONCLUSIONS
In conclusion, the addition of CPP to pep11 sequence allowed an efficient synthesis of a soluble peptide, which was able to penetrate into the cancer cells and to reduce their proliferation, both of which are encouraging characteristics for a possible anti-cancer usage.

HLA-G MEDIATED SUSCEPTIBILITY TO HPV-RELATED CERVICAL LESIONS

Presenter: Patricia De Araujo Souza

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BACKGROUND-AIM

Cervical lesions caused by high-risk human papillomavirus (hrHPV) remain a significant public health issue in low- and middle-income countries. Host immune responses influence the progression of HPV infection to cervical intraepithelial neoplasia (CIN). The HLA-G molecule is involved in immune tolerance and tumor evasion, thus, we aimed to investigate the association between HLA-G polymorphisms and high-grade cervical lesions (HG-CIN) in a cohort of Brazilian women.

METHODS

A case-control study was conducted, including 270 women with HG-CIN (CIN II/III) and 176 controls without cervical lesions. HLA-G genotyping and HPV detection and typing were performed by PCR-based methods. Odds ratios (OR) and respective 95% confidence intervals (95% CI) for multivariate analysis were calculated by unconditional logistic regression.

RESULTS

HPV16 was the most prevalent type in both cases (63.7%) and controls (11.3%). Nor were the 14 base pairs insertion in the 3' UTR region of HLA-G or the two-field HLA-G alleles associated with cervical lesions. However, the HLA-G*01:04:04 allele was associated with HG-CIN (OR=4.4, 95% CI=1.8-12.5, p=0.003). Interestingly, the HLA-G*01:04:01 allele which has the same predicted amino acid sequence, was not. The sequence difference between these alleles is a synonymous substitution of guanine to adenine at codon 267 in HLA-G*01:04:04, creating a predicted cryptic exonic splicing acceptor site by in silico analysis, which may influence mRNA splicing and protein expression.

CONCLUSIONS

The findings suggest that specific HLA-G alleles, particularly HLA-G*01:04:04, may influence susceptibility to HPV-related cervical lesions, potentially through mechanisms involving mRNA splicing and protein expression. This highlights the importance of genetic factors in cervical cancer development and underscores the need for further research into the functional consequences of HLA-G polymorphisms.

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THE PHILIPPINE CASE: VIRAL INFECTION REMAINS THE MAJOR RISK FOR LIVER CANCER
Presenter: Cyrollah Disoma

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BACKGROUND-AIM
Globally, liver cancer is the 6th most prevalent cancer type, and is ranked 3rd in terms of mortality rate. In the Philippines, it is the 4th most frequently diagnosed cancer and the third leading cause of cancer-related death. In 2022, nearly 9000 new cases and 12000 deaths were reported. Hence, liver cancer remains a major public health concern that requires attention.
METHODS
Chronic viral infections caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) are the predominant risk factors in the country. This is in contrary in the global statistics where MASLD is now considered as the major risk factor.
RESULTS
The Philippines has one of the highest prevalence of HBV in the Western Pacific region, with an estimated 8-12% of the population chronically infected. The common underlying causes include vertical transmission, limited vaccination coverage among older population, and inadequate access to antiviral therapy. HCV infection, although less prevalent, is also a significant concern, especially among high-risk population. In the Philippines where other viruses are less communicated to the public, coupled with poor lifestyle choices, exacerbate the liver cancer risk. Despite the national vaccination programs and efforts to improve screening and treatment, there are barriers that hinder the effective eradication of the disease. These include healthcare disparities across economic groups, low awareness, and limited access to preventive measures and antiviral therapy.
CONCLUSIONS
Addressing these challenges through enhanced public health strategies, increasing vaccination turn-out against HBV and HCV, and improved access to antiviral therapy is crucial in reducing the liver cancer incidence in a low-to-middle income country like the Philippines. As such, the country needs more trained personnel that can help in awareness and advocacy campaigns.

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LINE-1 METHYLATION IN OROPHARYNGEAL CANCER: THE INTERPLAY OF HPV-INFECTION AND TP53 MUTATION

Presenter: Elisabetta Fratta

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BACKGROUND-AIM

Although LINE-1 hypomethylation was found to represent a negative prognostic factor in oropharyngeal squamous cell carcinoma (OPSCC), the correlation between LINE-1 methylation and the status of HPV, p16 and TP53 is still largely unknown.

METHODS

Since 2019, an ongoing prospective study has been enrolling patients with OPSCC in nine cancer centers in Northern Italy. HPV genotyping and LINE-1 methylation were performed by real-time PCR. TP53 exon mutations were analyzed by NGS.

RESULTS

We evaluated 95 patients for HPV subtype, p16 over-expression, LINE-1 methylation, and

TP53 mutational status. The majority of cancers were diagnosed in the tonsil (n=48, 50.5%) or base of tongue (n=31, 32.6%). 21 patients (22.1%) were p16-/HPV-, 13 (13.7%) were p16-/HPV+, 60 (62.2%) were p16+/HPV+, and only one case was p16+/HPV-. In p16-/HPV+ patients, HPV was detected at a mean cycle threshold (CT) of 38.2 (min-max: 32.1-42.3) compared to a mean ct of 24.7 (17.2-32.2) among p16+/HPV+ ones. Among p16+/HPV+ patients, HPV16 was the most frequent genotype (n=52, 86.7%). TP53 mutation was found in 34 patients (35.8%), being more frequent in p16- patients (88.2%). Mean LINE-1 methylation was 30.5% in p16-/HPV- patients, 35.2% in p16-/HPV+, and 56.3% in p16+/HPV+ ($p<0.01$). Hypomethylation was reported in patients carrying TP53 mutation (31.0%) compared to wild type (56.6%; $p<0.01$). Interestingly, TP53 mutation exerted an hypomethylation effect in p16- patients (mean LINE-1 methylation: 28.4% and 61.8% in mutated and wild type patients, respectively; $p<0.01$).
CONCLUSIONS
This study identifies a remarkable fraction of p16-/HPV+ patients; however, HPV was detected at very high CT, suggesting that HPV was not casually involved in the etiology of these tumors. Both p16 and TP53 mutations influenced LINE-1 methylation, but TP53 mutations seems to have a role in LINE-1 methylation only in p16- OPSCC.

ELEVATING ANTI-TUMOR IMMUNITY: TARGETED DELIVERY OF AN ADENOVIRUS CANCER VACCINE VIA EXTRACELLULAR VESICLES IN A HUMANIZED MELANOMA MODEL
Presenter: Mariangela Garofalo

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BACKGROUND-AIM
Malignant melanoma is increasing globally. In Europe, registered cases rose from 146,321 in 2022 to an estimated 173,900 by 2025. Advanced cases have low survival rates, particularly those with NRAS gene mutations, which are more aggressive and have a median progression-free survival of only 4.8 months. Currently, there are no FDA or EMA approved treatments targeting this mutation. Oncolytic viruses show promise by selectively destroying cancer cells and stimulating immune responses, but their systemic use is hampered by antiviral immunity, making intratumoral administration necessary for better efficacy.
METHODS
In this study, we propose the systemic administration of a novel adenovirus-based cancer vaccine complexed in extracellular vesicles (EVs), naturally occurring nano-to-micron-sized delivery vehicles, to achieve a targeted therapeutic effect for hard-to-access tumors needing systemic therapy. This vaccine consists of an oncolytic adenovirus, Ad5/3-D24-ICOSL-CD40L,

and melanoma antigens that target NRAS mutations to enhance anti-cancer effects. We first tested the antineoplastic effect in three-dimensional co-culture models based on NRAS-mutated melanoma cells and peripheral blood mononuclear cells. Then, we established a humanized NRAS-mutated melanoma mouse model to explore the systemic delivery of the vaccine via EV formulations.
RESULTS
In vivo and ex vivo imaging analysis demonstrate the selective ability of EVs to deliver the oncolytic vaccine to both primary and metastatic tumors. The observed anticancer efficacy was attributed to reduced tumor volume and increased infiltration of tumor-infiltrating lymphocytes, including activated cytotoxic T-cells (GrB+CD8+). These findings align with the observed synergistic anti-tumor effect. Additionally, a correlation between tumor volume and activated CD8+ lymphocytes was observed.
CONCLUSIONS
Collectively, this research suggests EVs as a safe and effective strategy for the precise delivery of cancer vaccines in hard-to-reach NRAS melanoma tumors.

EPIGENETIC ALTERATIONS IN HPV+ TUMORS
Presenter: Lavinia Ghiani

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BACKGROUND-AIM
Head and Neck Squamous Cell Carcinomas (HNSCC) are classified into two subtypes: the HPV+, driven by human papillomavirus (HPV) infections, and the HPV-, linked to environmental risk factors. Although exhibiting distinct molecular and clinicopathological features, both lack effective tailored therapies. HPV-induced tumorigenesis is mainly driven by E6/E7 oncoviral proteins which also affect the epigenetics of the host cell. Epigenetic alterations are a hallmark of cancer and their characterization in HPV+ and HPV- HNSCC is crucial to identify both novel subtype-specific biomarkers and targets.
METHODS
Super-SILAC Mass Spectrometry was used to analyze the histone post-translational modifications of HPV+ and HPV- HNSCC samples and of E6/E7 overexpressing human primary keratinocytes. RNA-seq, RT-qPCR, Western blot analysis and immunohistochemistry were used to investigate the histone modifiers deregulated by E6/E7 in HNSCC samples. shRNA transduction in HNSCC cell lines, followed by RNA-sequencing and functional assays were used to investigate the oncogenic role of the selected target, specifically in HPV+ samples.

RESULTS
We found that the histone methyltransferase NSD2 and its target, H3K36me2, are upregulated in HPV+ HNSCC cell lines and patient samples compared to HPV- HNSCC and normal tissues. High-risk HPV E6/E7 oncoproteins, also increase their expression, indicating a key role for NSD2 in HPV-driven tumorigenesis. Our results show that NSD2, by conceivably reshaping H3K36me2 profiles, exerts a crucial oncogenic role in HPV+ and HPV- HNSCC, regulating both common and different pathways according to the subtype. We focused on the HPV+ subtype and found that especially there NSD2 is crucially implicated in the regulation of epithelial cell differentiation.
CONCLUSIONS
Our findings highlight H3K36me2 as a potential biomarker for patient stratification and NSD2 as a promising therapeutic target across HNSCC subtypes, regulating both shared and subtype-specific oncogenic pathways. In HPV+ HNSCC, NSD2 inhibition may restore epithelial differentiation and arrest tumor progression.

POLYCOMB GROUP PROTEINS PROTECT LATENT KAPOSI SARCOMA-ASSOCIATED HERPESVIRUS FROM EPISOME CLEARANCE AND HUSH-DEPENDENT CHROMATIN SILENCING

Presenter: Adam Grundhoff

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BACKGROUND-AIM
Kaposi sarcoma-associated herpesvirus (KSHV) is the causative agent of a number of human neoplasms. KSHV causes lifelong chronic infections by establishing latency reservoirs in which the viral double-stranded DNA genome persists as an extrachromosomal episome. We have previously shown that newly infecting episomes rapidly attract Polycomb Repressive Complexes 1 and 2 (PRC1, and PRC2, respectively), likely via CpG island features exposed by the epigenetically naïve viral DNA molecule. Here, we have used a series of genetic deletion models to dissect the functional interdependence of PRC1 and PRC2 during latency establishment.
METHODS
We employed individual and combined genetic deletion models of human PRC1, PRC2, and HUSH and investigated their functions in KSHV transcriptional regulation, lytic reactivation, and maintenance of latent episomes by comprehensive RNA-seq, ChIP-seq and biochemical analyses.

RESULTS
We identify KSHV genomes as a prime target for independent as well as collaborative de novo PRC recruitment and show that loss of PRC functions leads to severely altered chromatin states of viral episomes. We further demonstrate that PRC-mediated gene repression not only prevents aberrant transcription of viral genomes, but also protects episomes from detection by the human silencing hub (HUSH). In addition, we show that, unexpectedly, PRC complexes play an important role in ensuring episomal maintenance in dividing cells.
CONCLUSIONS
Taken together, our results demonstrate that the absence of PRC functions creates a metastable state of KSHV latency and implicate early chromatinisation as an integral step in latency establishment. Given the substantial fraction of herpesvirus genomes that exhibit CpG island features, we expect that the mechanisms described here also play an important role in the life cycle of other members of the family.

INFLUENZA VIRUSES CO-EXPRESSING BPV1 E6 AND E7 PEPTIDES TARGET BPV1 INFECTION UNDERLYING EQUINE SARCOID DISEASE, THUS SIGNIFICANTLY REDUCING TUMOUR RECURRENCE FOLLOWING THERAPY

Presenter: Edmund Karl Hainisch

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BACKGROUND-AIM
Papillomavirus (PV)-induced tumours continue to threaten the lives of human and animal patients. Targeting PV infection in a treatment regime may improve the response to therapy. Live-attenuated influenza viruses co-expressing bovine papillomavirus type 1 (BPV1)-specific E6 and E7 peptides as an immunotherapeutic vaccine were shown safe and well tolerated in healthy horses. Repeated intralesional injection with the vaccine lead to complete (CR) or partial sarcoid regression (PR) in 62% of horses in a first clinical study.
METHODS
To improve response in horses with severe and/ or multiple sarcoids, and to study the effect on BPV 1/2 infection, we started a large clinical study eventually comprising 100 horses. Mild, moderate, or periocular sarcoids are again treated by repeated intralesional injections. Horses with severe disease (large and/or multiple sarcoids) receive a combination of immunotherapy and electrosurgical tumour excision..

RESULTS
Nine of 21 horses treated conservatively so far showed CR six months after start of treatment. Importantly, all 7 samples collected from former tumour in sites in 7 horses tested BPV1/2-negative. Further 9 horses exhibited PR that is still ongoing. Three horses showed stable disease (SD). Samples from tumours still harboured BPV1 or 2. In one SD horse, surgery and booster injections led to freedom from sarcoids and BPV1 infection.
From 10 severely affected horses (64 tumours), nine showed CR six months after combination therapy. Three recurrent lesions in two horses were eradicated by repeated immunotherapy or combined therapy. This led to 8/9 horses being tumour- and BPV1/2-free.
CONCLUSIONS
Flu-BPV1-E6E7-immunotherapy had a response rate of 86% in mild and moderate cases. BPV infection was consistently eradicated in horses with CR. Combination therapy led to CR in 9/10 cases and significantly reduced recurrence, likely because of eradication of BPV1/2 infection. Only 3/64 treated sarcoids recrudesced, corresponding to a uniquely low recurrence rate of 4.7%.

IDENTIFICATION OF SIGNALING PATHWAYS INVOLVED IN RECK REGULATION BY HPV AND EBV ONCOPROTEINS

Presenter: Beatrice Jorge

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BACKGROUND-AIM
Cancers caused by High-Risk Human Papillomavirus (HR-HPV) and Epstein-Barr Virus (EBV) infection exhibit alterations in the levels and activity of matrix metalloproteinases (MMPs) and their negative regulator, RECK. Our research indicates that HPV E6/E7 oncoproteins downregulate RECK, and overexpression of RECK can reduce the tumorigenic potential of HPV-transformed cells. Similarly, EBV LMP1 also represses RECK expression. However, the mechanisms by which HPV and EBV oncoproteins regulate RECK are not well understood. Here, we investigated different signaling pathways altered by HPV and EBV proteins potentially involved RECK's modulation.
METHODS
We conducted an in silico analysis using three different and free access transcription factor's (TFs) prediction tools: PROMO-ALGGEN, JASPAR and Alibaba2 software. Cell lines: PHK, NP69,

HEK293 and HaCaT were transduced with retroviral vector carrying EBV LMP1 and HPV16 E6 and E7 sequences. Additionally, cervical cancer cell lines SiHa and SW756 were treated with chemical inhibitors targeting the STAT3 (NSC 74859) and NF-kb(JSH-23) signaling pathways. Total protein was then extracted to determine RECK's levels by Western Blot. Total RNA was also extracted for determination of viral oncogene's expression by polymerase chain reaction (PCR).
RESULTS
We analyzed the minimal RECK promoter sequence and identified four TFs with a high probability of binding site: AP1, PEA3, C/EBPA and SP1. We observed that EBV and HPV oncogenes expression was associated with altered RECK levels. Moreover, STAT3 and NF-kB inhibition led to a dose-dependent increase in RECK protein levels.
CONCLUSIONS
Alterations of RECK levels is a common property of HPV and EBV oncogenes. We identified TFs potentially involved in the regulation of RECK expression. Pharmacological inhibition assays indicate that STAT3 and NF-kB regulated pathways may be important for RECK regulation.

TRIPLE LYSINE AND NUCLEOSOME BINDING MOTIFS OF THE VIRAL IE19 PROTEIN ARE REQUIRED FOR HUMAN CYTOMEGALOVIRUS S PHASE INFECTIONS
Presenter: Rob Kalejta

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BACKGROUND-AIM
Tumor virus genomes are maintained as extrachromosomal plasmids within the nuclei of infected cells. When infected cells divide, the viral genome is at risk of being lost to the cytoplasm during mitosis because karyokinesis (nuclear division) requires nuclear envelope breakdown. Oncogenic viruses avoid genome loss during mitosis by tethering their genomes to cellular chromosomes, thereby ensuring viral genome uptake into newly formed nuclei. These viruses use viral proteins with DNA- and chromatin-binding capabilities to physically link viral and cellular genomes together in a process called tethering. The known viral tethering proteins of HPV (E2), EBV (EBNA1), and KSHV (LANA) each contain two independent domains required for genome tethering, one that binds sequence-specifically to the viral genome and another that binds to cellular chromatin. This latter domain is called a chromatin tethering domain (CTD).
METHODS
The human cytomegalovirus (HCMV) UL123 gene encodes a CTD that is required for the virus to productively infect dividing fibroblast cells within the S phase of the cell cycle, presumably

by tethering the viral genome to cellular chromosomes during mitosis. The CTD-containing UL123 gene product that supports S phase infections is the IE19 protein.
RESULTS
Here we define two motifs in IE19 required for S phase infections, an N-terminal triple lysine motif and C-terminal nucleosome binding motif (NMB) within the CTD.
CONCLUSIONS
HCMV modulates the properties of Glioblastoma Multiforme (GBM) tumors. Therefore, defining the mechanism through which IE19 tethers viral genomes to cellular chromosomes may help us understand tumor virus genome tethering and ultimately combat or control HCMV oncomodulation.

HUMAN PAPILLOMAVIRUS CAPSOMERS AS VACCINE PLATFORM FOR PROPHYLACTIC AND THERAPEUTIC HUMAN PAPILLOMAVIRUS ANTIGENS
Presenter: Ecem Kaplan

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BACKGROUND-AIM
Human papillomavirus (HPV) capsid protein L1 forms capsomers, which are pentameric subunits and can assemble into virus-like particles (VLPs). Chimeric HPV VLPs or capsomers have potential to serve as scaffolds for HPV E6, E7, E2 or L2 epitopes. Modifications of L1 protein to produce chimeric particles often interfere with the proper assembly or they result in low product yield. In the presented study, it is aimed to generate a modular L1-based scaffold to be decorated with HPV L2 and E7 epitopes. A protein glue, DogTag/DogCatcher, was used as a tool for decoration of capsomers.
METHODS
DogTag, 23 amino acids long peptide, was inserted into L1 protein and DogCatcher was fused to L2 and E7-based antigens. Both components were purified from E.coli. Once DogTag and DogCatcher partners are mixed, an isopeptide bond formation occurs between tag and catcher. Capsomers decorated with L2 and E7-based antigens were tested for prophylactic and therapeutic purposes, respectively.
RESULTS
We show that, Insertion of DogTag into the L1 protein do not disrupt the capsomer structure and the tag is accessible to react with DogCatcher partners. Decoration of capsomers shields the L1 epitopes so that the vaccination of mice with decorated capsomers induces only L2-

specific antibodies with titers that are significantly higher than those induced by monomeric form of the antigen. Mice immunized with the capsomers decorated with E7 antigen exhibits E7 specific T cell response whereas it is not significantly improved compared to the control antigen and it requires further optimization.
CONCLUSIONS
These findings demonstrate that HPV capsomer is a promising vaccine platform and besides, the modular DogTag-capsomers can be further investigated as a platform to display foreign antigenic epitopes.

HARNESSING ONCOLYTIC ADENOVIRUSES FOR MESOTHELIOMA TREATMENT: PRECLINICAL INNOVATIONS
Presenter: Lukasz Kuryk

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BACKGROUND-AIM
Viruses play a dual role in cancer biology, acting as oncogenic agents and therapeutic tools. While the role of oncogenic viruses in tumorigenesis is well understood, recent advances have focused on engineered viruses for cancer treatment. Oncolytic virotherapy, which uses viruses to selectively infect and destroy tumor cells, offers a promising therapeutic avenue. This study investigates an engineered oncolytic adenovirus designed to enhance immune responses in mesothelioma, a cancer with poor prognosis and limited treatment options.
METHODS
An oncolytic adenovirus expressing co-stimulatory molecules (CD40L and ICOS ligands) was combined with immune checkpoint inhibitors (ICIs) to boost anti-tumor efficacy. Preclinical studies included 2D and 3D in vitro systems and mesothelioma xenograft models. Tumor-specific replication, transgene expression, immune activation, and therapeutic outcomes were assessed using transcriptomic and immune assays.
RESULTS
The oncolytic adenovirus selectively replicated in tumor tissues, inducing localized transgene expression and robust immune activation. Combination therapy reduced tumor volumes and increased tumor-infiltrating lymphocytes (TILs). Transcriptomic analysis revealed upregulation of immune-related pathways and chemokines in the tumor microenvironment, reduced mesothelin levels, and evidence of immunogenic cell death. Adaptive immune response pathways were enriched, confirming the approach's immunostimulatory potential.

CONCLUSIONS
Engineered viruses show promise in cancer immunotherapy. Combining oncolytic virotherapy with ICIs offers a novel strategy for mesothelioma treatment, leveraging viral oncolysis and immune modulation to improve outcomes. Clinical translation of oncolytic viruses is crucial for maximizing their therapeutic impact.

INTERACTIONS BETWEEN MURINE POLYOMAVIRUS T ANTIGENS AND PP2A AFFECT VIRAL GENOME REPLICATION AND PACKAGING

Presenter: Erika Langsfeld

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BACKGROUND-AIM
Murine polyomavirus (MuPyV) sT is necessary for efficient genome replication and progression of viral DNA from sites of replication to sites of repair. sT and MT bind to host protein phosphatase 2A (PP2A) to control processes supporting viral replication. This interaction relies upon residues in sT which are important for efficient viral DNA synthesis. We hypothesized that mutation of these residues would affect not only vDNA replication, but also recruitment of host proteins to replicating DNA, and packaging into mature capsids.
METHODS
Using iPOND (isolation of proteins on nascent DNA), we compared the proteome bound to wild-type (WT) and sT/MT deletion mutant vDNA during and 2 hours after replication. To define functional domains in sT/MT mediating the activities of PP2A, we generated viruses harboring point mutations, singly and in combination. Binding strength between mutant sTs and PP2A was explored in silico. Phenotypic analyses included total genome replication, encapsidated genome production, and immunofluorescence.
RESULTS
iPOND data analysis revealed that while newly synthesized WT vDNA was bound to host replication proteins during active replication, most were not present on vDNA 2 hrs post-synthesis. In contrast, sT/MT deletion mutant vDNA was bound to many of the same replication proteins 2 hrs post-synthesis. Analysis of replication and genome packaging revealed defects across mutants, which correlated with predicted binding strength deficits between mutant sTs and host PP2A. Preliminary data suggests that the deletion mutant has a defect in recruitment of pATM to sites of viral replication.

CONCLUSIONS
These results suggest a stall in replication and/or repair when sT/MT are not present. Single and combination point mutant virus data support this assertion and suggest that subtle changes in the binding strength between sT/MT and PP2A lead to defects in genome replication and packaging. PP2A might function by recruiting ATM and other targets to sites of viral replication.

FIRST-VOID URINE SAMPLING FOR THE STUDY OF THE LOCAL HPV IMMUNE RESPONSE
Presenter: Marijana Lipovac

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BACKGROUND-AIM
Systemic immune responses to human papillomavirus (HPV) have been extensively studied, but the local HPV immune response and its effect on HPV infection remain less understood. Analyzing secretions collected with first-void urine (FVU) samples provides insight into (long-term) local HPV-specific immunity, including antibody (Ab) levels, functionality and infection dynamics. This approach may help close key knowledge gaps, such as the long-standing search for a correlate of protection.
METHODS
We measured HPV16 Ab levels in FVU and serum samples from two cohorts (n=58, 3 timepoints; n=50) using a Dissociation-Enhanced Lanthanide Fluorescent Immunoassay. Neutralizing capacity was assessed in the n=50 cohort using an FVU-optimized pseudovirion (PsV)-based neutralization assay. To study antigen-Abs interaction, we optimized a virion isolation protocol using FVU samples spiked with HPV PsV. Ongoing experiments aim to isolate WT-viral particles from women with an active HPV infection for use in a cell-based infection model to assess the impact of vaccine-elicited Abs on infectivity.
RESULTS
Results show that HPV16 Abs are detectable up to 3.5 years, and even 12 years, post-vaccination with strong correlations between FVU and serum (Rs=0.72; Rs=0.91, respectively). Additionally, we demonstrated that local HPV16 Abs, collected with FVU, retain their neutralizing capacity. Beyond Abs, we successfully isolated spiked HPV16 PsV from FVU samples, which remained infectious. First results of our HPV infection model will be presented, where we used HPV16 positive FVU samples to isolate virions.
CONCLUSIONS
We have shown that FVU is a reliable sample type for studying local HPV-related immunity. Immunoassay optimization will streamline its use in large-scale vaccine monitoring studies. Future research will optimize and validate an infection model using HPV16 positive FVU samples, comparing RT qPCR and BaseScope readout methods. These results may expand our knowledge of vaccine impact on the natural history of current infections.

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RESOLUTION OF A RECURRENT INVASIVE HPV19-RELATED CUTANEOUS SQUAMOUS CELL CARCINOMA AFTER RESTORATION OF T-CELL RECEPTOR SIGNALING
Presenter: Andrea Lisco

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BACKGROUND-AIM
Cutaneous squamous cell carcinoma (cSCC) is primarily caused by ultraviolet (UV) radiation-mediated oncogenesis, while β-human-papillomaviruses (HPVs) are considered facilitators dispensable for cSCC maintenance. We report on the first case of recurrent unresectable invasive cSCC associated to -HPV19 genomic integration and present the immunological context in which it developed as well as its clinical management.
METHODS
RNA Seq-based HPV genotyping of cSCC and next generation DNA sequencing-based were performed. A single base substitution (SBS) analysis of the tumor genome was performed to quantify the UV-induced mutation signature in the index case. The SBS profile was compared to other cases of sporadic (n=25) and recessive dystrophic epidermolysis bullosa (n=15) associated cSCC. ZAP70 biochemical activity and β-HPV19 T cell responses were analyzed before and after hematopoietic cell transplantation (HCT) in the proband.
RESULTS
We found that recurrent benign and malignant HPV-related diseases occurred in the context of pathogenic variants in ZAP70, an adaptor required for T-cell receptor (TCR) signal transduction. The molecular analysis of the recurrent invasive cSCC identified high-level expression of HPV19 genes including the E6/E7 oncogenic transcripts, in the context of viral genomic integration. The cSCC had a modest UV-associated mutational signature and lacked any specific mutation in cSCC driver tumor-suppressor genes. After engraftment of a ZAP70-wildtype haploidentical donor, the function and integrity of TCR signaling was restored with normalization of T-cell activation/proliferation, robust expansion of HPV19-specific T-cell responses and stable regression of cSCC.
CONCLUSIONS
Restoring TCR-signaling integrity by HCT led to the resolution of all benign and malignant HPV-related diseases revealing a direct role of β-HPV in skin carcinogenesis in hosts with defective adaptive T-cell responses.

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THE INTERACTIONS OF HUMAN PAPILLOMAVIRUS E6 AND E7 ONCOPROTEINS WITH CELLULAR PARTNERS AS TARGETS FOR DEVELOPING NOVEL THERAPEUTIC STRATEGIES AGAINST HPV-INDUCED CANCERS

Presenter: Arianna Loregian

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BACKGROUND-AIM
High-risk human papillomaviruses (HR-HPVs) are responsible for cervical cancers as well as tumors in the head-and-neck and anogenital regions. The carcinogenic potential of HR-HPVs is mostly related to two viral oncoproteins, E6 and E7, which represent potential drug targets. We report the characterization of small-molecule inhibitors of protein-protein interactions involving E6 and E7 with the aim of providing novel therapeutic approaches to treat HPV-associated cancers.
METHODS
Anti-E6 and anti-E7 compounds, selected from in silico screenings of small-molecule libraries, were tested by MTT assays in different HPV+ and HPV- cell lines. The ability of test compounds to inhibit the E6/p53 or E7/PTPN14 and E7/pRb interactions was determined through ELISA-based or cell-based assays. The rescue of cellular targets in cells treated with test compounds was assessed by Western Blot. The effects of compounds on apoptosis induction and cell cycle progression were assessed by FACS analysis. The compounds' antitumoral properties were also characterized through other cell-based assays. The binding of compounds to E6 or E7 was assessed through microscale thermophoresis.
RESULTS
Some of the test compounds specifically affected the viability of HPV+ cells. In addition, these compounds were able to restore p53, PTPN14 or pRb protein levels in HPV+ cells in a dose-dependent manner. Anti-E6 and anti-E7 compounds also inhibited the E6/p53 and E7/PTPN14 or E7/pRb interactions, respectively. Treatment of HPV+ cells with test compounds induced apoptosis while inhibiting the migration of HPV+ cells and could affect the viability of cell lines transformed by different HR-HPV genotypes (HPV16, 18, 45, and 68), suggesting a broad-spectrum activity. Finally, we could demonstrate the physical binding of some compounds to E6 or E7.
CONCLUSIONS
We report several protein-protein interaction inhibitors that directly target the E6 and E7 oncoproteins, paving the way for the development of specific anti-HPV therapies.

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IDENTIFICATION OF HIGHER RISK HPV16 SUBLINEAGES FROM ANAL SWAB WITH NEXT GENERATION SEQUENCING IS ASSOCIATED WITH INCREASED ODDS OF ANAL DYSPLASIA
Presenter: Derek Macmath

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BACKGROUND-AIM
High-risk human papillomaviruses (HPV) are the main cause of anal dysplasia and cancer. Although current anal cancer screening guidelines include HPV16 molecular testing in the algorithm, HPV16 sublineages have differences in their oncogenic potential for cervical cancer.
METHODS
Eighty adult participants (median age 36, 68% HIV+) underwent anal swab with cytology and next generation whole genome HPV sequencing (NGS). DNA extracted from swabs was sequenced and mapped to HPV16 reference genomes. HPV16 sublineages A1, A2, A4, C1, D2, and D3 were considered higher-risk and A3, B1, B2, B3, B4, C2, C3, C4, D1, and D4 were considered lower/unknown risk. Dysplasia was defined as ASCUS (atypical squamous cells of uncertain significance), LSIL (low-grade squamous intraepithelial lesion), or HSIL (high-grade SIL). Fisher's exact test was used to evaluate the association between higher-risk HPV16 sublineages and dysplasia.
RESULTS
Among participants, 35 (44%) were HPV16+. Of these, 16 (46%) had dysplasia (8 ASCUS, 8 LSIL). Nineteen (54%) had higher-risk (8 A1, 3 A2, 2 A4, 6 C1) and 15 (43%) had lower/unknown risk (2 A3, 10 B1, 3 B2) sublineages. One participant with A1 and B1 was considered higher-risk. Presence of a higher-risk HPV16 sublineage was associated with increased odds of dysplasia compared to a lower/unknown risk HPV16 sublineage (OR 6.96, 95% CI 1.29 – 51.87, p=0.016). High resolution anoscopy (HRA) was performed on 8 HPV16+ participants (7 higher-risk, 1 lower/unknown risk). Histology showed HSIL for 3 participants, all with high-risk sublineages, and was normal in the rest.
CONCLUSIONS
Sublineage identification with NGS is a promising modality to risk stratify patients with HPV16. In this cohort, presence of sublineages carcinogenic in cervical cancer was associated with anal dysplasia. This testing has the potential to reduce unnecessary HRAs in those with lower-risk sublineages and prioritize evaluation for those with the most cancerogenic strains.

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STIMULATING RESOLUTION OF INFLAMMATION TO UNLEASH ANTICANCER IMMUNITY IN HPV+ HEAD AND NECK CANCER

Presenter: Domenico Mattoscio

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BACKGROUND-AIM

Chronic non-resolving inflammation is a major hurdle to overcome for a successful antitumor response, as it induces an immunosuppressive tumor microenvironment (TME). Recent evidence suggests that inflammatory signals released in the TME alter the actions of key anticancer effectors, i.e. CD8 T cells, and promote their differentiation into a dysfunctional, exhausted phenotype (Tex). Thus, re-invigorating resolution (the ideal outcome of inflammation) with mediators including resolvins (RvD) could shape the plasticity of CD8 T cells and restore their antitumor functions.

METHODS

To address this, we used HPV+ head and neck cancer as an experimental system. Bioinformatic analysis of TCGA patient tumors, coupled with in vitro, in vivo, and omics approaches were used to dissect how restoring resolution reinvigorate T CD8.

RESULTS

In patients, Tex were significantly enriched in cytokine pathways, suggesting that inflammatory programs drive T CD8 antitumor functions. Exposure to RvD5 reduced cancer cell proliferation by enhancing T CD8 cell activity in vitro. Mechanistically, RvD5 stimulated T CD8 to block cancer cells in G2/M phase and downregulated markers of exhaustion in both CD8+ T (PD-1) and tumor cells (PD-L1). In vivo, RvD5 reduced cancer growth by decreasing the inhibitory PD-L1/PD-1 axis in the TME. Cytokine analysis, lipidomic and RNAseq revealed increased production and release of mediators involved in the antitumor response in the TME of RvD5 treated mice. Importantly, RvD5 increased the response rate of mouse tumors to anti-PD-1 treatment, suggesting that shifting cancer inflammation toward resolution is beneficial to enhance the efficacy of currently used immune checkpoint inhibitors.

CONCLUSIONS
Thus, RvD5 reduces tumor growth by modulating inflammation and delaying Tex in the TME. Therefore, inflammatory signals in the TME are crucial to shape T CD8 actions against cancer cells and their targeting could represent a new therapeutic strategy to potentiate antitumor immunity.

THE ROLE OF VAGINAL AND PERIPHERAL CYTOKINE PROFILES IN THE RISK OF CERVICAL INTRA-EPITHELIAL NEOPLASIA AMONG WOMEN COINFECTED WITH HIV/HPV

Presenter: Yanga Mdleleni

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BACKGROUND-AIM
Persistent genital infection with HR-HPV is associated with malignant transformation, and cervical intraepithelial neoplasia, which can become invasive cancer if untreated. Treatment failure and recurrence of CIN ranges between 20% and 75% among women living with HIV, relative to approximately 18% in HIV-negative women. Host factors are critical in regulating HPV-induced pre-malignancy, and cytokines that modulate immunologic control may be of particular importance. This study aimed to characterize the role genital and systemic cytokine profiles in the risk of CIN recurrence in South African women
METHODS
WLHIV aged 18-65 were followed up 1 year after CIN treatment. HPV genotyping and HR-HPV viral load were measured using Roche linear array and HC2 assay. While cytokines at baseline and 1 year after treatment were measured using multiplex ELISA
RESULTS
Over 70% of women in this cohort were HR-HPV+. Additionally, there were differences in the genital and peripheral cytokine profiles before and after treatment in these women, with plasma levels of proinflammatory cytokines significantly lower than genital cytokine levels ($p=0.001$*). Moreover, HR-HPV viral load was significantly associated the recurrence of cytokines in CVL and not in plasma. Furthermore, plasma cytokine profiles of IL-7, IL-8, and PDGF-bb in the Recurrence group, and cytokine profiles of IL-7, IL-8, and IL-9 in the No-recurrence group were significantly associated with hr-HPV viral load.

CONCLUSIONS
There were differences between genital and peripheral cytokine responses in women living with HIV, who were treated for CIN. Additionally, cytokine responses were associated with oncogenic HPV viral load before and after CIN treatment. They also suggest the potential practicality of the cytokine assays and HPV viral load for determining the prognosis of patients treated for CIN, especially women living with HIV

IN SV40, EARLY AND LATE TRANSCRIPTION ARE DIFFERENTIALLY CONTROLLED BY DIRECTIONAL REPRESSION FROM THE CENTRAL REGULATORY REGION

Presenter: Barry Milavetz

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BACKGROUND-AIM
Bidirectional transcriptional regulatory regions such as those typically found in DNA viruses, potentially can transcribe in either direction but typically only do so in one direction at a time. This raises the question whether transcription in the wrong direction is actively repressed by chromatin structure.
METHODS
In order to test for repressive chromatin structure, we have used a ChIP/ChIP-Seq strategy to determine the organization of chromatin in SV40 chromosomes primarily transcribing late genes. In the first ChIP we used antibody to RNAPII to immune-select for transcribing SV40 chromosomes. The bound transcribing chromosomes were fragmented and the released chromatin fragments subjected to a second ChIP with antibody to modified histones, followed by sequencing and bioinformatic analyses. .
RESULTS
When chromatin from transcribing SV40 chromosomes was analyzed, we found H3K9me1 to be present in 67+29% of the chromatin primarily at the early start and miRNA sites, while H3K9me3 was found in 14+11% of the transcribing chromosomes primarily at the miRNA site and the late start sites. Acetyl-H3 and acetyl-H4 were present in 1.8+1.5% and 5.4+6% respectively and were both present at the miRNA site, early start, and late start. The presence of H3K9me1 over the early start site was shown to depend upon T-antigen using the mutant SM virus which lacks a functional miRNA and over-produces T-antigen. We are presently determining whether this repressive chromatin structure is introduced during replication using a a similar approach with antibody to PCNA, a factor specific to replication, to immune select actively replicating SV40.

CONCLUSIONS
Our results suggest that chromosomes transcribing late genes are repressed for early transcription by the presence of a nucleosome containing H3K9me1 located at the start of early transcription, while chromosomes transcribing early genes are repressed for late transcription by a nucleosome containing H3K9me3 located at the start of late transcription.

EVALUATING THE ROLE OF A DNA METHYLATION READER, MBD2, IN DEVELOPMENT AND PROGRESSION OF HPV (+) AND (-) HNSCC
Presenter: Izge Shanlitourk

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BACKGROUND-AIM
Head and neck squamus cell carcinomas (HNSCC) are the seventh most prevalent malignancy worldwide. The majority of the patients suffer from poor prognosis, and those with better response often encounter life-long side effects. DNA methylation is an epigenetic modification that is disrupted in cancers. Methyl-CpG Binding Domain Protein 2 (MBD2) is an epigenetic reader which exhibits a critical role in regulating the gene expression by recognizing methylated cytosines and recruiting chromatin remodelling complexes. Studies show that MBD2 is critical in processes during differentiation, cancer and metastasis. A critical factor in HNSCC is epithelial to mesenchymal transition (EMT), which underlies key changes in cell states during carcinogenesis. To address this, we investigated MBD2's role in cancer progression of HPV+/- HNSCC.
METHODS
We generated transient MBD2 knockdown in HPV+/- HNSCC cell lines followed by downstream functional assays. Next, we investigated the MBD2 controlled transcriptional program in HPV+/- HNSCC.
RESULTS
TCGA datasets suggest that MBD2 expression is increased in HNSCC compared to normal tissues. Following this, we found that acute inhibition of MBD2 led to distinct phenotypes in HPV+/- HNSCC cell lines. MBD2 knockdown reduced clonogenic abilities of HPV- HNSCC cell lines and reduced cell migration. Consistent with these findings, RNAseq confirmed that PI3K, MAPK, and TGF-β pathways were significantly down regulated in HPV- cell line. Interestingly, we found that knockdown of MBD2 in HPV+ did not significantly affect cell viability and had minimal effects on transcription. Moreover, TGF-β addition restored the MBD2 knockdown phenotype in HPV- cell lines, increasing migration.

CONCLUSIONS
A key factor in the recurrence, metastasis, and resistance to therapy in HNSCC is the plasticity of the malignant cells, particularly through the process of EMT transition. These results are intriguing from a therapeutic perspective, and merit significant follow up.

KEY ASPECTS OF PAPILLOMAVIRUS INFECTION INFLUENCE THE HOST CERVICOVAGINAL MICROBIOME IN A PRECLINICAL MURINE PAPILLOMAVIRUS (MMUPV1) INFECTION MODEL
Presenter: Megan Spurgeon

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BACKGROUND-AIM
Human papillomaviruses (HPVs) are the most common sexually transmitted infection in the United States and are a major etiological agent of cancers in the anogenital tract and oral cavity. Growing evidence suggests changes in the host microbiome are associated with the natural history and ultimate outcome of HPV infection. We sought to define changes in the host cervicovaginal microbiome during papillomavirus infection, persistence, and pathogenesis using the murine papillomavirus (MmuPV1) cervicovaginal infection model.
METHODS
Cervicovaginal lavages were performed over a time course of MmuPV1 infection in immunocompetent female FVB/N mice and extracted DNA was analyzed by qPCR to track MmuPV1 viral copy number. 16S ribosomal RNA (rRNA) gene sequencing was used to determine the composition and diversity of microbial communities throughout this time course. We also wanted to determine whether specific microbial communities exist across the spectrum of MmuPV1-induced neoplastic disease. We, therefore, performed laser-capture microdissection to isolate regions of disease representing all stages of neoplastic disease progression (normal, low- and high-grade dysplasia, and cancer) from female reproductive tract tissue sections

from MmuPV1-infected mice and performed 16S rRNA sequencing.
RESULTS
Consistent with other studies, we found that the natural murine cervicovaginal microbiome is highly variable across different experiments. Despite these differences in initial microbiome composition between experiments, we observed that MmuPV1 persistence, viral load, and severity of disease influenced the composition of the cervicovaginal microbiome.
CONCLUSIONS
These studies demonstrate that papillomavirus infection can alter the cervicovaginal microbiome. Future studies will investigate how changes in the cervicovaginal microbiome influence papillomavirus pathogenesis and disease.

IDENTIFICATION OF DISEASE-ASSOCIATED POLYOMAVIRUSES FROM CYNOMOLGUS MACAQUES UNDERGOING STEM CELL TRANSPLANTATION
Presenter: Gabriel Starrett

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BACKGROUND-AIM
During immune suppression in humans, severe disease can be caused by the uncontrolled replication of normally dormant polyomaviruses. Mauritian cynomolgus macaques (MCM, Macaca fascicularis) are a highly inbred population of cynomolgus macaques that serve as important models for human immune responses in disease and transplantation due to their limited genetic diversity. Like humans, MCMs undergoing hematopoietic stem cell transplantation (HSCT) can develop hemorrhagic cystitis and nephropathy. These diseases have been associated with polyomaviruses by PCR, but the total diversity and genetics of these viruses has not been evaluated.
METHODS
We performed sequencing of rolling circle amplified urine DNA of 16 MCM that had undergone HSCT and 42 control animals of various geographic origins. The sequencing was then de novo assembled and annotated for viral sequences. Select samples that gave incomplete viral genomes were further evaluated using long read sequencing of virus amplicons.
RESULTS
We identified three distinct polyomaviruses in these animals. MafaPyV2 is the closest relative to BKPyV known to date and can be classified into 3 genotypes. SV40 type IIB has 97% nucleotide identity to SV40 type II, which has only been identified in Chinese rhesus macaques.

MafaPyV2 and SV40 type IIB was found 1.5-2.4 times as frequently in HSCT animals compared to controls. MafaPyV3 is closely related to WU and KI polyomaviruses, identified in two animals, and is the first of this polyomavirus species identified outside of humans.
CONCLUSIONS
We identified three polyomaviruses that appear to have specific host tropism to cynomolgus macaques including a new strain of SV40. All three viruses were detected more abundantly in immunosuppressed animals, with MafaPyV2 and SV40 type IIB identified in diseased tissues. These viruses offer opportunities to study disease during HSCT from viruses very closely related to those in humans in primate physiology and has potential for evaluating vaccines.

NUCLEAR HPV16 E2 FOCI FORMATION PRECEDES LATE E4 PROTEIN EXPRESSION
Presenter: Frank Stubenrauch

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BACKGROUND-AIM
In undifferentiated keratinocytes, HPV genome replication is limited, with only early viral gene expression occurring. Productive replication begins in suprabasal layers, where infected cells re-enter the S-phase of the cell cycle. In G2, activation of the differentiation-dependent viral late promoter triggers genome amplification and expression of the late E4 protein, followed by capsid protein expression. The E2 activator and E8^E2 repressor regulate transcription and genome replication in basal keratinocytes, but their roles in productive replication remain unclear.
METHODS
Immunofluorescence analyses of HPV16-pos. cells
RESULTS
Using a novel polyclonal serum and monoclonal antibody 22E2-B9, both of which detect HPV16 E2 and E8^E2 proteins in immunofluorescence assays, nuclear E2 accumulations are identified in a subset of monolayer cells harboring replicating HPV16 wt) or E8^E2 mt (E8-) genomes. Wt cells primarily show 1–2 E2 foci, while E8- cells display multiple foci. Co-localization with known E8^E2 interactors suggests that wt E2 foci contain both E2 and E8^E2. Additional co-localization with replication markers (RPA32, RAD51, ©H2AX, Brd4) and cell cycle markers indicates that these foci are viral replication centers, forming in S-phase and persisting until G2, marking the onset of the productive phase.

Interestingly, only some E2 foci-positive cells express E4, though this increases with methylcellulose-induced differentiation. Consistently, E2 foci-positive/E4-negative, E2 foci-positive/E4-positive, and E2 foci-negative/E4-positive cells appear in the suprabasal layers of organotypic cultures, the gold standard for modeling the HPV replication cycle.
CONCLUSIONS
These findings suggest that the formation of E2-positive viral replication centers is an early event in the differentiation-dependent productive replication cycle, preceding E4 protein expression. Moreover, the disappearance of E2 foci in E4-positive cells implies that E2 accumulation is tightly regulated throughout the productive phase.

DEVELOPMENT OF A VIRUS-POSITIVE MODEL OF METASTATIC MERKEL CELL CARCINOMA FOR PRECLINICAL STUDIES
Presenter: Monique E. Verhaegen

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BACKGROUND-AIM
Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine skin cancer often accompanied by integration of Merkel cell polyomavirus DNA, driving expression of viral small T antigen (sTAg) and a truncated large T antigen (tLTAg). Using CreER- and doxycycline-inducible transgenes expressing sTAg and tLTAg, along with the transcription factor ATOH1 (to drive Merkel cell fate), and deletion of Trp53, we generated the first mouse model of MCC (SLAP mouse). We now aim to advance this MCC model for preclinical studies.
METHODS
We generated modified SLAP mice with full Trp53 deletion and induced transgenes in neonatal mice to yield MCC skin tumors. We established the first mouse MCC cell line from this model and assessed tumorigenicity and metastasis in immune competent C57BL/6J mice via intradermal or tail vein injections. Proof of concept treatment studies on allografted mice were performed with anti-PD-1 and/or a lysine-specific histone demethylase 1 inhibitor (LSD1i).
RESULTS
Modified SLAP mice developed cutaneous tumors with 100% penetrance and lymph node involvement. Tumors resembled virus-positive human MCCs and expressed MCC markers SOX2, ISL1, KRT8 and KRT20 in a dot-like pattern, and synaptophysin. The SLAP cell line produced macroscopic MCC allografts in C57BL/6J mice and expressed all MCC markers. Allografted mice developed regional lymph node metastases and tail vein injections yielded

liver and lung metastases. Treated allograft tumors resulted in a 4.6 (vehicle), 2.3 (anti-PD-1), and 1.1 (LSD1i) -fold increase, or a 1.1 (combination) -fold decrease in average tumor volumes compared to pre-treatment volumes, and smaller lymph node metastases. All experimental groups displayed an increase in F4/80+ macrophages and CD8+ T cells.

CONCLUSIONS

This model is the first and only model of virus-positive MCC that yields local tumors as well as regional and distant metastases in immunocompetent mice, paving the way for much-needed translational studies for this aggressive tumor.

SIRT1 TARGETING AS A POTENTIAL RADIOSENSITIZING STRATEGY IN HPV-ASSOCIATED CANCER

Presenter: Marta Catozzo

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BACKGROUND-AIM

The pathogenesis of HPV-associated cancers is driven by the dysregulated expression of the viral oncoproteins E6 and E7, which target p53 and pRb for proteasomal degradation. We recently discovered a novel mechanism by which high-risk HPVs (e.g., HPV16) suppress p53 activity, centered on the cellular deacetylase SIRT1 (Lo Cigno et al., 2023). Our findings indicate that SIRT1 inhibition restores the transcriptionally active K382-acetylated form of p53 in HPV+ cell lines, reducing cell survival and clonogenicity compared to HPV- cells. Furthermore, treatment with EX527, a SIRT1 inhibitor, sensitizes HPV+ cells to genotoxic agents. In this study, we explore the anticancer effects of SIRT1 inhibition combined with radiotherapy in HPV+ cell lines.

METHODS

Using flow cytometry, cell viability assays, and colony formation assays, we evaluated the potential radiosensitizing effects of SIRT1 inhibition by EX527 in HPV+ cells. The combined treatment is also being assessed in a C3.43-based mouse tumor model of HPV16-driven cancer.

RESULTS

NOKE6/E7 (stably transduced with HPV16 E6 and E7 oncogenes), CaSki (HPV16+ cervical carcinoma), and SCC152 (HPV16+ head and neck carcinoma) cell lines were treated with EX527 for 24 hours before exposure to low doses of ionizing radiation (IR). The combination of EX527 and IR significantly enhanced the anticancer effects of radiation, as demonstrated by MTT viability assays. This enhancement was not observed in HPV- head and neck carcinoma-derived HNO150 cells, which harbor a mutated p53. In colony-forming assays (CFA), EX527 pre-treatment notably reduced colony formation compared to vehicle-treated cells, indicating an irreversible effect. Flow cytometry analysis revealed a significant increase in cell cycle arrest only in HPV+ cells following exposure to 1Gy combined with SIRT1 inhibition, compared to either treatment alone.

CONCLUSIONS

Our findings suggest that combining EX527 with IR could represent a promising therapeutic strategy for HPV-related cancers.

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HPV INTEGRATION AND CHROMSOMAL INSTABILITY IN HPV-INDUCED TUMORIGENESIS
Presenter: Yoke-Chen Chang

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BACKGROUND-AIM
Human papillomaviruses (HPV) are oncogenic viruses responsible for 5% of cancers globally, including cervical, vulvar, vaginal, penile, and head and neck cancers. Current triage for cancer risk assessment relies on high-risk HPV genotyping but lacks effective tools to stratify tumorigenesis. HPV integration plays a key role in the progression of squamous lesions.
METHODS
We hypothesized that a high-sensitivity molecular suite based on HPV integration into the host genome could aid in stratifying at-risk women with HSIL. We developed a custom hybridization capture assay (JUNC-SEQ) detecting all known 220 HPV types, offering comprehensive HPV typing and revealing HPV-DNA insertions positions, orientations, and structures.
RESULTS
We identified specific integration patterns, including “heterocatena”, where HPV DNA appears extrachromosomal or as tandem repeats within the human genome. We developed junction FISH (JUNC-FISH) to visualize HPV integration, enabling examination of clonal expansion at the single-cell level in HSIL and in cervical and vulvar tumors. AI-guided histopathology identify tissue features linked to HPV-induced genomic instability (e.g abnormal mitosis, cellular pleomorphism) in HPV-positive lesions. The AI-driven histopathology correlated with HPV integration mapping improves HPV associated cancer progression risk prediction. We observed cells with high copy HPV DNA hubs along the basement membrane. These findings highlight features of chromosome instability, genomic instability, and clonal heterogeneity.
CONCLUSIONS
Our custom developed JUNC-SEQ, JUNC-FISH assays and AI-enhanced histopathology show promise in identifying lesions based on integrated HPV presence and high HPV DNA copy numbers. Our work emphasized the importance of molecular tools in understanding HPV-related carcinogenesis and enhancing cervical cancer risk assessment, providing insights into mechanisms of chromosome instability induced by HPV and their implications in tumor progression.

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ONCOGENIC PROPERTIES OF THE E6 AND E7 PROTEINS OF G-1 HUMAN PAPILLOMAVIRUS TYPE 4

Presenter: Paola Di Bonito

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BACKGROUND-AIM
The genus gamma (©) comprises the largest group of viruses within the PV family, including more than 300 genotypes. ©HPVs have been detected in cutaneous and mucosal epithelia of healthy and cancerous tissues. However, their direct role in the development of neoplasia remains to be established. In a previous comprehensive investigation, we detected a large spectrum of ©HPVs in both healthy-looking (HS) and lesional skin of patients affected by Actinic Keratosis (AK), and found that ©1-HPV4 was more prevalent in AK than in HS.
We investigated the oncogenic properties of ©HPV4 E6 and E7 in a primary human foreskin keratinocyte (HFK) experimental model.
METHODS
The HPV4E6 and E7 open reading frames were cloned into a retroviral vector. Recombinant retroviruses were generated in packaging cells and then used to transduce primary HFKs. Cell proliferation, expression levels of factors associated with the p53/pRb pathways, and the miRNAs induced by E6 and E7 were analysed.
RESULTS
An immortalised HFK line expressing the HPV4-E6 and E7 genes was established and compared (K4) to HFKs immortalised with the E6 and E7 genes of ‹7 HPV16 (K16) and ®2 HPV38 (K38). The HPV4-E6 and E7 genes altered the transcription of TERT, cyclin A, cdk2, and cdc2 mRNAs, as well as the expression levels of p53, p21, Gadd45, E2F1, cyclin A, p16INK4a, cdk2 and p16INK4a proteins. The analysis of 384 miRNAs in K4 compared to HFKs has shown modulation of miRNAs involved in tumorigenesis, similar to that observed in K16 and K38.
CONCLUSIONS
Overall, ©1-HPV4 can modulate key factors of cellular growth and cycle control, and miRNA regulation may be a mechanism through which gamma HPV E6 and E7 proteins promote oncogenesis. The data reveal that HPV4 shares certain oncogenic mechanisms with HPV16 and HPV38.

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APOBEC3A/B--DEPENDENT ONCOGENE REGULATION IN HPV-INDUCED CELL TRANSFORMATION
Presenter: Gangupam Bhavani

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BACKGROUND-AIM
Approximately 24 high-risk HPV types cause human cancers, such as cervical cancer and head and neck squamous cell carcinoma (HNSCC). APOBEC3 (A3) proteins, initially known for antiviral activity, are implicated in cancer development. A3A and A3B exhibit high mutational burdens in HPV-related cancers. Our study aims to explore A3 proteins' editing-independent role in HPV infection and cell transformation, focusing on oncogenes regulated by A3A or A3B in HPV-positive cells.
METHODS
Methods involved in silico gene expression analysis of cervical cancer and HNSCC samples, complemented by RNASeq of human foreskin keratinocytes (HFK) and HPV16-positive HFK16 cells. We selected oncogenes correlating with A3A or A3B for biochemical and functional studies, examining protein-protein interactions, cellular localization, and transformation potential.
RESULTS
In silico gene expression analyses identified a number of oncogenes and keratinocyte differentiation genes whose expression was largely correlated with the expression of the A3B protein. Among the oncogenes, CDKN2A (encoding p16INK4A) was identified as a promising candidate, as it is a well-established biomarker for cervical cancer and HPV-positive HNSCC. Overexpression of A3B protein reduced CDKN2A protein levels and cell migration of HeLa cells, while the intracellular localisation of CDKN2A was not altered. Our data suggest that non-editing functions of APOBEC3 proteins play an important role in HPV-induced cell transformation, at least in part through the differential expression of host oncogenes.
CONCLUSIONS
Our study highlights APOBEC3B's non-editing role in HPV-related cell transformation, focusing on oncogene regulation, such as CDKN2A. A3B overexpression leads to decreased CDKN2A protein levels, suggesting a mechanism for A3B's involvement in cancer progression. These findings provide new insights into the non-catalytic functions of A3 proteins in HPV-related cancers, prompting further investigation.

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NSD2 IN CERVICAL AND HEAD AND NECK CANCERS AND ITS ROLE ON THE REGULATION OF EPITHELIAL DIFFERENTIATION
Presenter: Farkhondeh Ghoryani

F. Ghoryani
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BACKGROUND-AIM
Persistent infection with high-risk human papillomaviruses (hrHPV), including HPV16 and HPV18, is the most important risk factor for cervical cancer (CC) and a subgroup of head and neck cancer (HNC). To induce carcinogenesis, E6/E7 viral oncoproteins interfere with important tumour suppressor pathways, p53 and pRB, leading to uncontrolled cell proliferation. These oncoproteins also influence host epigenetic regulators. NSD2, a histone methyltransferase, implicated in several cancers, remains poorly characterized in CC and HNC. We investigated NSD2 levels in HPV+ CC and HNC and how NSD2 regulates epithelial cell differentiation in both tumors.
METHODS
We examined NSD2 expression in human primary keratinocytes (HKs) upon HPV16/18 E6/E7 overexpression both in CC and HNC cell lines. Furthermore, NSD2 expression in patient samples was examined by analyzing RNA-seq TCGA data from the OncoDB database for both tumors. NSD2 levels were also assessed in HPV+ and HPV- HNC by RNA-seq on laser captured FFPE patients' samples. The impact of NSD2 on epithelial differentiation was assessed by analysing the differentiation markers expressions upon NSD2 overexpression or silencing in HKs and HPV+ cell lines.
RESULTS
NSD2 function in HPV-driven transformation was confirmed by its upregulation in HPV16/18 E6/E7-expressing keratinocytes and its downregulation upon E6/E7 silencing in HPV+ cell lines. Furthermore, RNA-seq data on laser captured FFPE HNC samples showed higher levels of NSD2 in HPV+ patients compared to HPV- ones, consistent with observations in HNC cell lines. Also, TCGA data, revealed higher NSD2 levels in HPV+ HNSCC relative to the HPV- cases, and higher levels of NSD2 in all CC cell lines compared to the normal ones. Elevated NSD2 in CC regardless of HPV status suggests additional regulatory mechanisms.
NSD2 silencing in HPV+ cell lines of both tumors, resulted in ΔNp63α downregulation, an oncogene and master regulator of epithelial differentiation, and in the upregulation of multiple differentiation markers.
CONCLUSIONS
We found that E6/E7 oncoproteins play a key role in NSD2 upregulation and that NSD2 contributes to the epithelial cell differentiation regulation. Therefore, targeting NSD2 may be a potential therapeutic approach to restore differentiation and arrest the development of CC and HPV+ HNSCC tumors.

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CHARACTERIZATION OF THE IMPACT OF HPV E7 ONCOGENE ON THE FUNCTIONS OF THE E3-UBIQUITIN LIGASE RNF168 AT STALLED REPLICATION FORKS.
Presenter: Ottilie Le Doeuff

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BACKGROUND-AIM
High-risk human papillomaviruses (HPV) are recognized etiological agents of around 4% of all cancers. The survival of HPV+ cancer cells is dependent on the ability of the viral oncoproteins E6 and E7 to respectively prevent apoptosis and promote cell-cycle progression. Our group showed that E7 modulates DNA repair pathway choice at DNA double-stranded breaks by interacting with the DNA repair factor RNF168. E7 interacts with the E7-binding domain (E7BD) of RNF168 and does not impair its E3-ligase activity. We reported that E7 colocalizes with RNF168 at sites of replication stress. This project aims at defining whether E7 affects the function of RNF168 upon replication stress.
METHODS
Our proximity-based ligation assay (BioID) enables to track the accumulation of RNF168 at stalled replication forks and map its replication-specific interactome. U2OS RNF168 KO clones expressing TurboID-RNF168 WT or ⊗E7BD are exposed to a short treatment of hydroxyurea (HU) prior to the addition of biotin for 20 min. The recruitment of RNF168 and known DNA repair factors is detected by quantifying their colocalization with foci labeled using fluorophore-tagged streptavidin. Streptavidin pulldown followed by mass spectrometry is used to reveal a snapshot of RNF168 interactors at stalled forks with or without expression of a GFP-tagged HPV16 E7.
RESULTS
Both WT RNF168 and ⊗E7BD are efficiently recruited at stalled forks upon treatment with HU. Strinkingly, only RNF168 ⊗E7BD blocks the recruitment of the BRCA1-A complex at stalled fork, suggesting a role of the E7BD in the regulation of DNA repair pathway choice at these loci. Here I will present our recent progress in characterizing this phenotype.
CONCLUSIONS
The proximity-based assay develop is a unique tool to study the impact of E7 on the replication-dependent function of RNF168. Importantly, our findings raise the possibility that E7 dictates the outcome of replication stress in HPV+ cancer cells by modulating RNF168 functions at stalled forks.

PD-L1 EXPRESSION BY MACROPHAGES AND MYELOID-DERIVED SUPPRESSOR CELLS IN THE TUMOR MICROENVIRONMENT OF EPSTEIN-BARR VIRUS-ASSOCIATED HODGKIN LYMPHOMA IN PEDIATRIC PATIENTS

Presenter: Paola Chabay

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BACKGROUND-AIM
In Argentina, Epstein-Barr Virus (EBV) is associated with Hodgkin Lymphoma (HL) in more than 70% of cases in children under 10 years. PD-L1, expressed by HRS and tumor microenvironment (TME) cells, plays a key role in promoting pro-tumoral conditions. PD-L1 overexpression was reported in the EBV+ TME of HL in pediatric patients, although the cell type involved remains unclear. Our aim was to assess whether MDSCs and macrophages, both components of the TME in adult cHL, express PD-L1 in pediatric EBV+ cHL and to compare its expression with EBV-infected tonsils.
METHODS
This study included 22 cHL and 36 tonsil formalin-fixed paraffin-embedded (FFPE) biopsies from pediatric patients. EBER in situ hybridization (ISH) was performed to assess EBV presence. Expression of CD33 (MDSC marker), CD68 and CD163 (macrophage markers), as well as PD-L1 expression, was analyzed by immunohistochemistry (IHC), and expressed as positive cells/mm .
RESULTS
Thirteen cHL patients were EBV- and 9 were EBV+. PD-L1+ cells were significantly higher in EBV+ patients ($p=0.0009$). No significant differences were observed for CD33+, CD68+, or CD163+ cells, or their co-expression with PD-L1, between EBV- and EBV+ cases ($p>0.05$). Tonsil analysis included 11 primary infected (PI), 10 carriers (C), 10 reactivated (R), and 4 non-infected (NI) patients. CD33+/PD-L1+ expression was significantly higher in C compared to PI, R, and NI ($p=0.0005$, $p=0.0164$, $p=0.0039$), whereas CD68+ and CD163+ (and their PD-L1 co-expression) did not differ significantly ($p>0.05$). Additionally, a significant difference in PD-L1 and PD-L1/CD163 expression was found between PI and R groups ($p=0.0274$, $p=0.0131$).
CONCLUSIONS
These findings suggest that while MDSCs and macrophages may not directly drive PD-L1 overexpression in HL's TME, PD-L1 upregulation in MDSCs could represent a mechanism for immune regulation in pediatric patients with persistent EBV infection.

DECIPHERING THE ASSOCIATION BETWEEN HPV ONCOPROTEINS AND POLO-LIKE KINASE 1
Presenter: Peter Ho Yin Luk

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BACKGROUND-AIM
Cervical and head and neck cancers can be caused by the infection of Human Papillomavirus type 16. It encodes viral oncoproteins E6 and E7 to manipulate the host cell cycle and contribute to cancer progression. Polo-like kinases (PLKs), a family of serine/threonine kinases, are key regulators of the cell cycle. Abnormal expressions of Plk1 are often observed in tumors, making it a promising target for cancer therapy. However, studies examining the association between HPV oncoproteins and Plk1, as well as the efficacy of Plk1 inhibitors in HPV-positive cancers, are lacking. Our study aims to study the interaction between HPV oncoproteins, E6 and E7 with the association of Plk1, and to study if blocking this interaction can stop HPV-associated cancer progression.
METHODS
Immunoprecipitation, co-immunoprecipitation and immunofluorescence were used to study the biochemical interaction between Plk1 and HPV-E6/E7. Western Blotting and gene silencing approaches were used to study the cell signalling pathways involved in the interaction between Plk1-E6/E7 leading to carcinogenesis. Cytotoxicity assay was performed on HPV-positive and negative cell lines to investigate the efficacy of Plk1 inhibitors on HPV-positive cancer. Additionally, the impact of Plk1 inhibition on cancer cell phenotypes was explored through cell proliferation, migration, invasion, and colony formation assays.
RESULTS
We reported that Plk1 is overexpressed in HPV-positive cancers. Pull-down assays and Co-Ip revealed that both HPV-16 E6 and E7 interact with Plk1. IF indicated that Plk1-E6 interaction occurs near the nucleus while Plk1-E7 interaction occurs in both the nucleus and cytoplasm. Depletion and inhibition of Plk1 lead to decreased E7 protein and restoration of pRB protein. Moreover, Plk1 inhibitors demonstrate greater selectivity and efficacy on HPV-positive cancer cells compared with HPV-negative cancer cells.
CONCLUSIONS
These findings suggest the drug-ability of Plk1 for HPV-related cancers.

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IMPACT OF EBV ON GENETIC MUTATIONS IN DLBCL: A STUDY EMPLOYING CONVENTIONAL AND HIGH-SENSITIVITY DETECTION METHODS IN A COHORT FROM ARGENTINA
Presenter: Paola Chabay

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BACKGROUND-AIM
Epstein Barr virus (EBV)+ Diffuse Large B Cell Lymphoma (DLBCL) is a new entity confirmed by WHO. EBV may act as an alternative or complementary mechanism to the genetic alterations involved in DLBCL. Since sensitive methods described traces of EBV infection in cases originally considered negative, the role of EBV in DLBCL pathogenesis is still under discussion.
This study aims to analyze the genetic alterations, classify them according to their pathogenicity, and correlate these with the presence of the virus and its traces.
METHODS
EBV+DLBCL was defined by EBERs in situ hybridization, with 20% of EBERs+ tumor cells as cut-off. Viral traces were analyzed using a ViewRNA assay to detect LMP1 and EBNA2 transcripts. Genetic variants were evaluated using a custom next-generation sequencing panel, focusing on pathogenic variants and pathway enrichment. Comparative analyses were conducted among EBV+ DLBCL, EBV- DLBCL with and without traces.
RESULTS
NGS analysis identified pathogenic variants mainly in ATM, TP53, PTEN, ARID1B, and KMT2A genes across all groups, suggesting shared mechanisms of DNA repair dysfunction and cell cycle regulation. No significant association was found between EBV presence or traces and the frequency of pathogenic variants when they were analyzed as a whole. However, EBV+ DLBCL exhibited unique alterations in BTK, HAX1, PAFAH1B1, and NAGLU genes, implicated in immune regulation, apoptosis, and mitochondrial dynamics, while 75% of EBV+ DLBCL displayed variations in C11orf65 gene. NOTCH pathway variants were exclusively enriched in EBV+ DLBCL.
CONCLUSIONS
EBV and its traces do not impact pathogenic variants in DLBCL, suggesting an epigenetic or immunomodulatory role in lymphomagenesis. Frequent mutations in ATM, TP53, and PTEN across all groups highlight genomic instability's role, while genetic alterations in specific genes and the NOTCH pathway in EBV+ DLBCL suggest virus-specific mechanisms (not mediated by traces) that may promote tumorigenesis.

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DECODING Y CHROMOSOME LOSS IN BOTH HPV+ AND HPV– HNSCC: UNVEILING Y-LINKED GENES'S ROLE IN TUMOR DEVELOPMENT
Presenter: Carolina Scagliusi

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BACKGROUND-AIM
Head and Neck Squamous Cell Carcinoma (HNC) is the sixth most common cancer worldwide, driven by environmental factors and human papillomavirus (HPV) infections.
Epidemiological studies consistently show a higher incidence of HNC in males than in females. Initially, this was attributed to higher smoking and alcohol consumption in males, but recent studies reveal that even in non-smokers and non-drinkers, males remain at higher risk, suggesting molecular and genetic factors contribute to susceptibility.
A key genetic difference between sexes is the presence of Y chromosome. In bladder cancer, Y chromosome loss and KDM5D downregulation are linked to increased tumor aggressiveness, and metastasis.
A TCGA study analyzing 12 cancers also found reduced expression of few Y-linked genes correlates with higher cancer risk in males.
Our study investigates the impact of Y chromosome alterations in male HNC patients, comparing HPV+ and HPV– to determine whether male susceptibility is common to both or specific to HPV– HNC.
METHODS
We performed RNA-sequencing on HNC FFPE tissue samples to characterize the transcriptional profiles. To assess the mRNA expression levels of Y-linked genes, we analyzed the TCGA, while RT-qPCR and WB to evaluate gene expression in HNC cells.
Shallow WGS on FFPE tumor samples from the same HNC patients will assess Y chromosome loss at the DNA level, with FISH validation of Y chromosome status.
To explore functional relevance, we will use shRNA/siRNA knock-down, CRISPR-Cas9 knock-out, and overexpression of target genes. Functional assays will evaluate tumorigenic potential.
RESULTS
RNA-seq analysis of FFPE HPV– vs. HPV+ tumors identified differential expression, with a subset of genes showing significant variation. Both TCGA data and qPCR analysis confirmed the downregulation of these genes in HPV– HNC patients.
CONCLUSIONS
In the future, assessing Y-linked gene expression may enable personalized therapies, improving patient selection for better therapeutic responses and survival.

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A COMPREHENSIVE REVIEW OF THE PRECLINICAL MODELS FOR ORAL POTENTIALLY MALIGNANT DISORDERS AND THEIR PROGRESSION TO ORAL SQUAMOUS CELL CARCINOMA
Presenter: Izge Shanlitourk

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BACKGROUND-AIM
Oral potentially malignant disorders (OPMDs) are classified as a group of oral lesions with the capacity to progress to oral squamous cell carcinoma (OSCC). There is a lack of understanding of the driving mechanisms of the OPMDs to OSCC transition. Although Human papillomavirus (HPV) is an established risk factor for oropharyngeal squamous cell carcinoma, its role in the pathogenesis of OSCC is limited. To decipher these underlying mechanisms, preclinical model systems, both in vitro and in vivo, are of vital importance. In this review, we have compiled a detailed summary of the available preclinical models used in OPMD research and their progression to OSCC including HPV related model systems.
METHODS
A consortium of pioneers in the field of OPMDs and OSCC performed an extensive research to accumulate available data from the literature to address key concepts such as identifying the strengths, limitations, and usage of the model systems.
RESULTS
An up-to-date compendium of the most recent literature on preclinical model systems to study and understand OPMDs and their progression to OSCC has been anthologized. We

have described the existing 2D and 3D in vitro model systems, both in unicellular forms, like spheroids, as well as multicellular forms, including organotypic raft cultures, which more accurately allow investigation of cell-to-cell crosstalk. We have also outlined the available in vivo model systems, including chemical carcinogen-induced models, xenograft models, syngeneic models, and genetically engineered mouse models, In addition, we have extensively discussed the presence of HPV in the OPMD pathogenesis and their progrerssion to OSCC.
CONCLUSIONS
Altogether, this review serves as an atlas of existing preclinical models for studying the development of OPMD lesions and their progression to OSCC. It highlights future directions, emphasizing the development of new models to enhance diagnostic and therapeutic strategies and ultimately improve care for patients with OPMDs.

TSPYV SMALL T ANTIGEN LEADS TO ACCUMULATION OF THE HAIR FOLLICLE SIGNALING EFFECTOR B-CATENIN IN TRANSGENIC MICE

Presenter: Li-Jyun Syu

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BACKGROUND-AIM
The polyomavirus TSPyV causes a rare hair follicle disorder called trichodysplasia spinulosa (TS). In patients with TS, hair follicles on the face and ears are enlarged, structurally abnormal, filled with viral particles, and produce spikes instead of hairs. The TSPyV genome encodes late as well as early viral proteins including small (sTAg) and large (LTAg) tumor antigens, which activate the host cell cycle machinery for viral genome replication. Although prior studies have examined responses to TSPyV TAgs in cell culture, our aim is to use transgenic mouse models to gain insight into how TSPyV disrupts hair follicle biology to cause disease.
METHODS
We chose to focus our efforts on TSPyV sTAg based on initial screening studies. To test the effects of sTAg on epidermal biology we generated 1) K5 promoter-driven mice constitutively expressing sTAg in skin epithelia, and 2) knock-in mice carrying Cre-inducible sTAg inserted into the ROSA26 locus.
RESULTS
Pre-term K5-sTAg embryos have a severe phenotype mimicking mice with skin-targeted b-catenin (b-cat), a major regulator of hair follicle morphogenesis and cell fate. K5-sTAg

mice contain high levels of b-cat in K5+ epidermal cells, leading to widespread activation of hair follicle lineage markers and repression of epidermal markers. Postnatal activation of sT expression leads to aberrant follicle growth, ectopic b-cat accumulation, and de novo hair follicle formation, a proposed mechanism underlying TS development. Immunostaining of human TS revealed ectopic expression of b-cat, reflecting our findings in sTAg mice.
CONCLUSIONS
TSPyV sTAg expression in mouse skin leads to accumulation of b-cat, a key regulator of hair follicle development. These findings are in keeping with the hypothesis that in addition to canonical functions of early viral proteins, TAgs may possess novel cell-type specific functions which operate to expand the number of host cells capable of supporting productive viral infection.

MODERATE SCRIB EXPRESSION LEVELS ARE ASSOCIATED WITH POOR PROGNOSIS IN OPSCC PATIENTS IRRESPECTIVE OF HPV STATUS
Presenter: Vjekoslav Tomaic

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Head and neck cancer ranks as the sixth most common cancer worldwide. Besides traditional risk factors like smoking and alcohol consumption, Human papillomavirus (HPV) infections are becoming increasingly important, particularly in Western populations. While HPV infection offers a significant survival advantage, the need for reliable biomarkers remains. Our study aimed to determine whether the expression levels of three PDZ domain-containing proteins (SCRIB, NHERF2, DLG1), known as HPV E6 cellular targets, affect the survival outcomes of HNSCC patients primarily treated with surgery (n=48). The samples included oropharyngeal and oral cancers, with HPV presence confirmed through PCR and p16 staining. Clinical data and follow-up records were sourced from the hospital database and the Croatian Cancer Registry up to November 2023. Survival analysis was performed using the Kaplan-Meier method and Cox proportional hazard regression. The results were validated by re-evaluating a comparable subset of TCGA cancer patients (n=391). Among the three targets studied, only SCRIB levels were identified as an independent predictor of survival in the Cox regression analysis, alongside

tumor stage. Further studies within a typical Western population context are needed, given the ongoing prevalence of smoking and alcohol consumption in Croatia. Notably, the strongest link between survival and SCRIB levels was found in HPV-negative cases.

COMPREHENSIVE ANALYSIS OF PANHPVAX-INDUCED IMMUNE RESPONSES
Presenter: Heiko Weyd

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BACKGROUND-AIM
Prevention against infection by human papillomaviruses (HPV) can be afforded by vaccination with the currently licensed HPV vaccines inducing neutralizing antibodies against the capsid protein L1. Implementation of HPV vaccination programs worldwide, however, is still a challenge due to vaccine-related costs and logistic requirements. To address this problem, we have developed a heat-stable, low-cost vaccine based on the minor capsid protein L2 (PANHPVAX), which is currently being tested in a phase I clinical trial. Key aspect of this novel vaccine are broadly protective humoral responses induced by PANHPVAX, driven by anti-L2 neutralizing antibodies. However, comprehension on innate immune responses and changes in B cell phenotype induced by PANHPVAX is lacking. Here, we follow-up on our prior observations by analyzing PANHPVAX-induced immunity with respect to L2-specific antibody profile as well as anti-L2-mediated effector functions such as cellular cytotoxicity and phagocytosis.
METHODS
We will analyze sera of PANHPVAX-vaccinated probands for antibody-dependent cellular cytotoxicity or phagocytosis by employing standardized cellular assays. PBMC of probands will be analyzed for the presence of immune cell subsets and their respective phenotypes by flow cytometry and single cell RNA sequencing.
RESULTS
Preliminary results have been obtained for cellular assays of antibody-dependent phagocytosis and cytotoxicity. Using differentiated THP-1 cells we show a time- and concentration-dependent uptake of antibody coated latex beads. Model antibodies against HeLa cell surface antigens mediate cytotoxicity by a human NK cell line.
CONCLUSIONS
Results obtained so far will guide us in establishing reliable cellular assays for the analysis of patient samples. A comprehensive analysis of humoral and cellular immune responses of probands in the PANHPVAX phase I trial will be essential to further improve the vaccine and to inform the next steps in the clinical development process.

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