Meeting report: Fourth Summer School on Innovative Approaches for Identification of Antiviral Agents (IAAASS).

APPENDICES

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Description : this document contains all the abstracts presented by the students that participated at the Fourth Summer School on Innovative Approaches for Identification of Antiviral Agents held from September 24 – 28 2018 in Santa Margherita di Pula, Italy.
The content is divided in three appendices:

APPENDIX A : SELECTED ORAL PRESENTATIONS

APPENDIX B : POSTER PRESENTATIONS

APPENDIX C : TRAVEL GRANT AWARDEES

APPENDIX A: SELECTED ORAL PRESENTATIONS

SMALL MOLECULES TARGETING COXSACKIEVIRUS A16 CAPSID INACTIVATE VIRAL PARTICLES AND PREVENT VIRUS BINDING

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Coxsackievirus A16 (CVA16) is an etiologic agent of hand, foot, and mouth disease (HFMD) that affects young children, and although typically self-limited, severe complications and fatal cases have been reported. Due to the lack of specific medication and vaccine against CVA16, there is a need to develop effective antivirals for better control of its infection in epidemic areas. In this study, we identified the tannins chebulagic acid (CHLA) and punicalagin (PUG) as efficient small molecules that can abrogate CVA16 infection in human rhabdomyosarcoma cells. Both compounds significantly reduced CVA16 infectivity at micromolar concentrations without apparent cytotoxicity. Mechanistic analysis revealed that the tannins particularly targeted the CVA16 entry phase by inactivating cell-free viral particles and inhibiting virus binding. Further examination by molecular docking analysis pinpointed the tannins' targets in the CVA16 capsid's 5-fold axis canyon region near the pocket entrance that functions in cell surface receptor binding. Intriguingly, the tannins also docked closely to the putative residues recognized for CVA16 interaction with cell surface glycosaminoglycans. We suggest that CHLA and PUG are efficient antagonists of CVA16 entry and could be of value as antiviral candidates or a starting point for further development against CVA16 infection.

IN-VITRO EVALUATION OF ANTIVIRAL COMPOUNDS SIMULTANEOUSLY INHIBITING ZIKV AND DENV REPLICATION USING AN ELISA CELL-BASED ASSAY

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Zika (ZIKV) and Dengue (DENV) viruses belong to the Flaviviridae family and are transmitted by mosquitoes of the Aedes genus. The World Health Organization has ranked DENV as the most critical mosquito-borne viral disease and declared ZIKV an international public health emergency. Despite intensive work, no specific antiviral therapy is available for ZIKV or DENV. The high degree of conservation of essential viral enzymes among Flaviviridae makes it reasonable to search for "broad-spectrum" antivirals. Efficient and fast cell-based assays are highly needed to test simultaneously candidate inhibitors of ZIKV and DENV replication.
A fast and reliable ELISA cell-based assay has been developed and applied to the screening of second-generation 2,6-diaminopurine derivatives targeting a conserved pocket on DENV/ZIKV polymerase, which is needed to bind helicase/protease and generate the functional replication complex. Following determination of the 50% cytotoxic drug concentration (CC50), the ELISA assay assessing drug concentration inhibiting 50% of viral replication (IC50) is completed in 5 days, compared to 8 days (ZIKV) and 13 days (DENV) of the reference plaque assay. All the 6 compounds investigated showed anti-ZIKV activity (median IC50 2.83 μM, range 0.4-9.5) while 5 compounds showed anti-DENV activity (median IC50 11.2 μM, range 0.9-12.5). The

best selectivity index (SI: ratio CC50/IC50), was obtained for compound MR323, which is more active against ZIKV with respect to DENV (SI 81.3 vs. 19.2, respectively). Thus, this class of compounds appears to be attractive and warrants further evaluation. Structural improvement of MR323 is underway to increase anti- DENV activity and progress towards a single compound inhibiting multiple viruses.

ANTIVIRAL EFFECTS OF CETYLPYRIDINIUM CHLORIDE OVER THE REPLICATION CYCLE OF HERPES SIMPLEX VIRUSES

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Herpes simplex viruses type 1 and type 2(HSV-1 and HSV-2) are pathogens that produce a wide spectrum of clinical diseases. Particularly, in the orofacial area, HSV-1 infects the mouth and the eye eliciting gingivostomatitis, lesions around the lips, conjunctivitis and keratitis, among others. Although most of these infections can be treated with acyclovir, its effectiveness is somewhat limited when applied locally. Hence, better topical antivirals against this virus would be desirable. Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound that is added to some types of mouthwashes and toothpastes as an antiseptic and has a broad antimicrobial spectrum. While bactericidal and fungicidal effects have been widely reported for CPC, an antiviral effect for this compound has only been recently described over influenza.
The aim of this study was to evaluate the antiviral effect of CPC over the replication of HSV in vitro and identify a possible mechanism of action. In our experiments, human gingival fibroblasts and epithelial cells treated with CPC displayed reduced infection with HSV strains that encode structural and non-structural versions of the green fluorescent protein (GFP). Our results suggest that CPC inhibits HSV replication and blockade occurs after the virus enters the cell, yet before viral genome replication takes place. Thus, CPC in topical formulations

ZIKA VIRUS NS2A INHIBITS INTERFERON SIGNALING AND IS A POTENTIAL TARGET TO RESTORE THE HOST IMMUNE RESPONSE

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Type I interferon (IFNα/β) response is crucial to restrain a number of pathogenic infections, activating a potent antiviral state in the host. Stimulation of IFN receptors (IFNAR1 and IFNAR2) leads to the activation of Jak1, Tyk2 and signal transducers of transcription (STAT1 and STAT2), resulting in the expression of the IFN-stimulated genes. Investigations into flavivirus-host cell interactions have identified a number of viral proteins counteracting this response. NS1, NS2B/3, NS2A, 2K-NS4B and NS5 are the principal proteins involved in IFN antagonism of flavivirus. Among them also Zika virus (ZIKV) has developed diversified strategies to evade the system. NS1, NS2A, NS2B, NS4A, NS4B, and NS5 are responsible for IFN production inhibition, while NS2B/3 and NS5 are known to inhibit IFN signaling. The high virulence of flavivirus infections is undeniably linked to immune evasion mechanisms. Studies on Kunjin virus have shown that a single amino acid substitution in viral protein NS2A (A30P) is responsible for the suppression of IFN-β transcription and resulted in diminished virulence in mice. We found that also NS2A of ZIKV counteracts the IFN response inhibiting IFN signaling. Immunofluorescence analysis showed that NS2A blocked STAT1 phosphorylation. Given the impact that the IFN antagonism has on flavivirus virulence, the knowledge gained by characterizing the mechanism through which ZIKV evades IFN is a pioneer in the

attenuation of the pathogenesis contributing to the development of countermeasures directed against novel attractive pharmacological targets as NS2A.

CHARACTERIZATION OF POST-TRANSLATIONAL MODIFICATIONS DURING HUMAN CYTOMEGALOVIRUS INFECTION: IMPLICATIONS FOR NOVEL ANTIVIRALS

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Human Cytomegalovirus (HCMV), a widespread β-Herpesvirus, establishes a lifelong latency in the myeloid lineage, with reactivation often driven by inflammation. Autoimmune diseases (AD) are characterized by chronic inflammation due to an abnormal immune response against the body's own tissues. In genetically predisposed patients, HCMV has been associated with AD, but whether it initiates or supports the development of AD is still not known. Citrullination is a post-translational modification (PTM) catalyzed by peptidylarginine deaminases (PAD) that convert peptidylarginine into peptidylcitrulline, whose dysregulation has been linked to a spectrum of ADs, cancer, and neurodegenerative disorders. Against this background, the goal of this project is to characterize citrullination during infection with HCMV, that may be relevant In the etiopathogenesis of AD. Here, we demonstrate that HCMV infection upregulates the overall pattern of citrullination in HFF (Human Foreskin Fibroblasts) using two different approaches: a specific antibody recognizing citrullinated residues and a citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe. Consistently, PAD2 expression increased both at the mRNA and protein levels, suggesting a predominant role for this isoform in HCMV-induced citrullination. Surprisingly, viral replication rate of the HCMV is strongly impaired in the presence of a specific pan PAD-inhibitor, indicating that citrullination is required for HCMV replication. By mass- spectrometry based-analysis, we observed antiviral proteins of the IFIT and Mx1 family to be significantly citrullinated at 48 hpi, suggesting that HCMV has evolved a strategy to evade the host's immune system by their citrullination-mediated inactivation. Based on our preliminary results, we hypothesize that altering HCMV-induced viral and /or cellular protein PTMs, which we show for the first time to be essential for HCMV replication, might represent an alternative strategy for efficient inhibition of HCMV viral replication even in the presence of drug resistance mechanisms due to viral DNA polymerase mutations, a severe complication in the treatment of HCMV disease. To conclude, these findings may shed light on an alternative antiviral approach and potentially elucidate the role of HCMV in the etiopathogenesis of ADs.

EXTRACT OF *PARTHENIUM HYSTEROPHORUS* LEAVES CONTAINS ANTI-HIV-RT POTENTIAL

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An estimated 36.7 million people including children were living with the HIV till 2016 with a prevalence of 0.8% among adults. Natural products have been found to inhibit enzymes and proteins crucial to the life cycle of HIV. The toxicity of currently available anti-HIV drugs makes it difficult to maintain patient's adherence to antiretroviral therapy, as a consequence, the search for better anti-HIV agents continues and focused on natural sources, particularly the plant species. Parthenium hysterophorus L. (Asteraceae) also known as congress grass, is an annual herb. The P. hysterophorus leaves collected were shade-dried and grinded into fine powder with mortar and pestle. The powdered leaves were sequentially extracted using hexane (HX), benzene (BZ), chloroform (CH), ethyl acetate (EA), acetone (AC), ethyl alcohol (ET), and water

(AQ) using Soxhlet apparatus. These extracts were centrifuged, filtered, and lyophilized. The dried residues were dissolved in DMSO. The reaction mixture consists of template/primer complex, dNTPs, and reverse transcriptase (RT) enzyme in lysis buffer with or without extract. After 1 h incubation at 37°C, the reaction mixture was transferred to streptavidin coated microtiter plate (MTP). The biotin-labeled dNTPs that are incorporated in the template due to activity of RT were bound to streptavidin. The unbound dNTPs were washed and anti- digoxigenin-peroxide (anti-DIG-POD) was added to MTP. DIG- labeled dNTPs incorporated in the template were bound to anti- DIG-POD antibody. The unbound anti-DIG-POD was washed and peroxide substrate (ABTS) was added to MTP. A colored reaction product was produced during the cleavage of substrate catalyzed by peroxide enzyme. The absorbance of the sample was measured at 405 nm using microtiter plate ELISA reader. The resulting color intensity is directly proportional to the actual RT activity. The percentage inhibitory activity of extracts was calculated by comparing to a sample that does not contain an inhibitor. The P. hysterophorus extracts were evaluated for antiretroviral activity by targeting HIV-RT enzyme using HIV-RT kit (Roche). Anti-HIV- RT activity was measured at two different concentrations (0.6 and 6.0 ??g/ml), which showed low inhibition potential (<50%) in vitro. About 40% inhibition of reverse transcriptase (RT) activity was observed in hexane fraction at 6.0 ??g/ml concentration. The study showed that phytochemicals present in P. hysterophorus leaf have the potential agents with low to moderate anti-HIV-RT activity. The extracts (HX, ET, and AQ) produced modest anti-HIV-RT activity (about 23–40%). Nevirapine, the standard anti-HIV drug, showed 99.67% inhibitory activity. Since several phytochemicals are present in the crude extract, it might be possible that isolation and purification of the active ingredients from potential fractions of HX, ET, AQ and their bioactivity testing in the future may provide further enhancement of anti-HIV-RT activity.

GIVING DRUGS A SECOND CHANCE: REPURPOSING HEPARIN AND ITS DERIVATIVES TO PREVENT ZIKA VIRUS-INDUCED CELL DEATH IN HUMAN THREE-DIMENSIONAL (3D) NEUROSPHERES (NS) DERIVED FROM PLURIPOTENT STEM CELLS

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The severe consequences of fetal ZIKA virus (ZIKV) infection have highlighted the need of antiviral agents for the treatment of infected pregnant women. One attractive approach for combating emerging and rapidly spreading infectious diseases is drug repurposing. Heparin, a widely used anticoagulant safely used in pregnant women, has antiviral activities against attachment and entry of several enveloped viruses. We explored the effects of heparin on ZIKV replication and cytopathic effects (CPE) in human neural progenitor cells (hNPCs) grown as neurospheres (hNS). Incubation of hNS with heparin (100 µg/ml) 1 h prior to ZIKV infection resulted in a modest but significant decrease of infectious virus release (by 5-10 fold); however, heparin fully prevented virus-induced CPE of hNS cultures. Moreover, heparin prevented the formation of intracellular vacuoles, a typical feature of paraptosis, together with the inhibition of HMGB1 release in hNS culture supernatant. De-sulfated heparin derivatives maintained heparin effects implying that sulfate groups are not critical for preventing virus-induced CPE. In summary, heparin could be potentially exploited as lead compound to discover novel agents for preventing virus replication and CPE.

HUMAN ENDOGENOUS RETROVIRUSES TRANSCRIPTIONAL ACTIVATION AND MODULATION IN PBMCs AFTER IN VIVO LPS STIMULATION

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About 8% of the human genome consists of Human Endogenous Retroviruses (HERVs), retroviral sequences integrated into the germline cells million-year-ago. There are several pieces of evidence regarding the modulation of HERV expression associated with the systemic modulation of the immune response. We investigated HERV expression in immune activation setting, by using an RNAseq workflow for the analysis of Differential Expression (DE) of HERVs. The public dataset used includes the transcriptome of Human Peripheral Blood Mononuclear Cells (PBMCs) from 15 healthy participants to a clinical trial assessing the RNA expression following the injection of 1ng/Kg LPS. We found that about the 7 % of these elements is transcribed in PBMCs. We identified 5040 differentially expressed HERV elements, of which 3583 were higher expressed after LPS stimulation and 1457 were higher expressed in not stimulated samples. HERV-Fb and HML-2 were the families that included the higher number of differentially expressed elements. We characterized the most differentially expressed HERVs by checking their genomic context of insertion. Interestingly, we found that between the 15 most differentially expressed HERVs, 11 elements integrated close to or within genes that are involved in the immune response, and their expression is modulated in the same way of those genes as consequence of LPS stimulation.
These data suggest that HERVs are modulated in inflammatory contexts and may interact with the immune host response.

GAT-2 AS AN ENTRY RECEPTOR FOR SEMLIKI FOREST VIRUS

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Host factors play crucial roles in virus infections. Viruses exploit various cellular processes and are counteracted by an arsenal of host antiviral defences. Characterisation of these interactions is crucial for understanding the viral life cycle and developing novel antiviral treatments. Semliki Forest virus (SFV) is a positive-strand RNA alphavirus that has been used as a model virus for multiple clinically significant diseases such as lethal encephalitis. We have conducted follow up studies on a previously performed genome-wide siRNA screen that revealed novel cellular genes affecting SFV infection. We used an automated high-throughput imaging-based approach combined with a previously developed endocytic bypass assay to pinpoint the role of these factors to early or later steps of SFV infection in HeLa cells. For comparison, we also analysed the effects of their depletion on the infectivity of vesicular stomatitis virus (VSV), a negative-strand RNA virus. We have identified the γ-aminobutyric acid (GABA) transporter, GAT-2, as a potential receptor for SFV. We have also show that TNP01, RPL18, ETF1 and GNDPA1 are required in the entry and membrane penetration steps of SFV infection. In addition, we have shown that DDX54 promotes SFV infection and EIF2B3, EIF4G1, PHB2, EDF1, and DDX47 counteract SFV infection in the later stages. Our results are a starting point for the further characterisation of alphavirus-host cell interactions, the understanding of which is crucial for the development of novel therapeutics.

TARGETING A HOST FACTOR TO CONTROL BOTH HUMAN VIRAL INFECTION AND INSECT VECTOR COMPETENCE: THE CASE OF THE HUMAN RNA HELICASE DDX3X AND THE RNA HELICASE BELLE OF AEDES ALBOPICTUS

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Human DEAD-box polypeptide 3 (DDX3X) is an ATPase/RNA helicase involved in the replication of many viral pathogens making it an attractive target for broad spectrum antiviral agents limiting the occurrence of drug resistance. We have previously demonstrated that DDX3X inhibitors can block the replication of different RNA viruses, thus demonstrating that the inhibition of a cellular cofactor essential for the viruses but not for the cell can represent a successful strategy for the development of novel antiviral agent.

DDX3X possesses a unique motif of ten residues that forms a helix that positions a positively charged loop in close proximity to the putative RNA ligand and not generally found in other human DExD-box helicases. This motif could be an important determinant in RNA substrate recognition/activation by DDX3X. We biochemically characterized this motif since it could be exploited as a new target for novel inhibitors developed in collaboration with the Department of Biotechnology, Chemistry and Pharmacy of the University of Siena. Targeting a very specific and conserved motif could improve the selectivity of our inhibitors for their target, thus avoiding side effects.

RNA viruses, like WNV but also DNV, are spread by arthropod vectors and can cause explosive outbreaks. Currently, there are no approved drugs or vaccines available to counter epidemics by these viruses. Human DDX3X is important for viral replication in the human host. We identified the RNA helicase Belle as a close ortholog of DDX3X present in *Aedes albopictus*, one of the main vectors of Flaviviruses. However, it is not known whether Belle plays any role in the vector competence for Flaviviruses replication and transmission. Since our ten-years' experience in the characterization of DDX3 protein, we start to first characterize Belle protein to understand if it could be manipulated to control vector competence**.**

EXPRESSION OF RECOMBINANT CONSERVED ANTIGENIC REGION OF HIGH RISK HUMAN PAPILLOMAVIRUS E7 ONCOGENE IN 293 CELL LINE

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Human papillomavirus (HPV) induced cervical cancer is the second most common cause of death in females after breast cancer. So far no therapeutic vaccine is available for HPV. Three prophylactic vaccines by Merck and GlaxoSmithKline have been confirmed to prevent high risk HPV strains but these vaccines have been shown effective only in the girls from 11 to 12 years which are not exposed to HPV previously. E7 protein of HPV is involved in altering immune response in patients by down regulating apoptosis. The proposed research plan involves isolation of HPV prevalent strains from cervical cancer tissue. After amplification and sequencing antigenic epitopes will be predicted and cloned in ubiquitous chromatin opening elements containing lentiviral vectors for stable transfection in 293 cell line. Using optimized lentiviral vectors, correctly folded and post translationally modified, endotoxin free recombinant immunodominant regions in E7 protein upto 20-100 mg/l can be achieved in conventional small scale (100 ml) culture. At these yields, most proteins can be purified using a single chromatography step, immediately. As these will be raised in mammalian cell lines with all the necessary post translational modifications and will be appropriate for use in therapeutic applications.

THE DEVELOPMENT OF ROTAVIRUS VACCINE PROTOTYPE BASED ON VIRUS-LIKE PARTICLES FORMED BY CHIMERIC PLANT VIRUS PROTEIN.

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Rotavirus is one of the main causes of viral gastroenteritis. Attenuated vaccines have decreased the level of rotavirus infection, but they have some disadvantages, like by-effects and high cost. The development of new effective and safe recombinant rotavirus vaccine is desirable. According to the literature data 14-amino acid length epitope (RLSFQLMRPPNMTP) of structural protein VP6 can induce the strong protective immune response. This is a promising antigenic determinant for rotavirus vaccine designing. At the same time using plant viruses as a base for vaccine development is a perspective approach, since plant viruses are absolutely safe for humans and have immunopotenting activity. Alternanthera mosaic virus (AltMV) (family *Alphaflexiviridae*, genus *Potexvirus*) coat protein (CP) can form virus-like particles (VLPs), which are stable under physiological conditions and enhance immune response. We designed the chimeric protein contains VP6 epitope fused to the C-term of AltMV CP. This protein was named ER6. ER6 had rotavirus antigenic specificity demonstrated with the commercial antiserum. *In vitro*-assembled ribonucleoprotein complexes (RNPs) consisting of viral RNA, AltMV CP and ER6 were obtained. The structure of these VLPs (RNPs) was analysed by electron microscopy. It was clearly demonstrated that not only individual ER6 but also ER6 within the RNP complexes (VLPs) has rotavirus antigenic specificity. This promising result provides evidence that ER6 and VLPs (RNPs), which contains it, can be useful for following recombinant rotavirus vaccine design.
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TORQUETENOVIRUS VIREMIA AS A MARKER OF IMMUNOCOMPETENCE IN LIVER OR KIDNEY TRANSPLANT RECIPIENTS.

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Torquetenovirus (TTV) is a highly prevalent, nonpathogenic, circular single-stranded human DNA virus. Previous studies indicated that TTV levels are elevated after solid organ transplantation and correlates with the extent of immunosuppression, however further investigations are needed to clearly understand its role as a marker of immunocompetence. TTV kinetics were studied by real-time PCR in 280 liver or kidney transplant recipients who underwent different drug regimens to maintain immunosuppression. Results: During one year post-transplant follow-up, TTV viremia fluctuated irrespective of transplanted organ type but in according to the immunosuppression regimen. To evaluate whether TTV levels may have a role in the prediction of the occurrence of viral complications, TTV loads of 69 patients who developed cytomegalovirus (CMV) infection pt were compared with those in which CMV reactivation did not occur. Plasma TTV load measured between day 0 and 10 post-transplant was significantly higher in CMV DNA positive than in CMV DNA negative patients. TTV viremia above 3.45 log DNA copies/ml within the first 10 days post-transplant correlates with higher propensity to CMV reactivation following transplantation. This study provides evidence for the potential use of early post-transplant TTV viremia to predict CMV reactivation in liver or kidney transplant recipients.

EVASION STRATEGY OF HUMAN CYTOMEGALOVIRUS FROM THE ANTIVIRAL ACTIVITIES OF APOBEC3G

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The apolipoprotein B editing enzyme catalytic subunit 3 (APOBEC3) is a family of DNA cytosine deaminases that mutate and inactivate viral genomes by single-strand DNA editing, thus providing an innate immune response against a wide range of DNA and RNA viruses. In particular, APOBEC3A (A3A), a member of the APOBEC3 family, is induced by human cytomegalovirus (HCMV) in decidual tissues where it efficiently restricts HCMV replication, thereby acting as an intrinsic innate immune effector at the maternal-fetal interface. However, the widespread incidence of congenital HCMV infection implies that HCMV has evolved to counteract APOBEC3-induced mutagenesis through mechanisms that still remain to be fully established. Here, we have assessed gene expression and deaminase activity of various APOBEC3 gene family members in HCMV-infected primary human foreskin fibroblasts (HFFs). Specifically, we show that APOBEC3G (A3G) and to a lesser degree A3F, but not A3A, gene products are upregulated in HCMV-infected HFFs. We also show that HCMV mediated induction of A3G expression is mediated by interferon-β (IFN-β), which is produced early during HCMV infection. However, knockdown or overexpression of A3G does not affect HCMV replication, indicating that A3G is not a restriction factor for HCMV. Finally, through a bioinformatics approach, we show that HCMV has evolved mutational robustness against IFN-β by limiting the presence of A3G hotspots in essential open reading frames (ORFs) of its genome. Overall, our findings uncover a novel immune evasion strategy by HCMV with profound implications for HCMV infections.

CHARACTERIZATION OF THE HUMORAL RESPONSE DIRECTED AGAINST HERPES SIMPLEX VIRUS GLYCOPROTEINS IN NATURALLY INFECTED PATIENTS

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Herpes Simplex Virus (HSV) infection can be particularly severe among immunocompromised, immunosuppressed patients or newborns leading to severe clinical sequelae. The current pharmacological therapy consists of drugs specific for herpes viruses but burdened by drug resistance or kidney toxicity. Furthermore, no vaccines are available to date. Hence, novel alternative anti-HSV compounds are needed. We identified a human monoclonal antibody (mAb#33) targeting glycoprotein D of HSV (gD) that proved to inhibit virus replication *in vitro* before as well as after HSV entry, even against HSV clinical isolates featuring different susceptibility to acyclovir. Then, *in vivo* studies demonstrated that a single intravenous administration performed after HSV-1 and HSV-2 ocular and vaginal lethal challenge fully protected mice from severe disease and death.

The viral gD is one of the most immunogenic HSV proteins able of eliciting both neutralizing and non-neutralizing Abs *in vivo*. Therefore, not all Abs naturally elicited by gD are able to protect from HSV infection. Moreover, the antiviral activity of neutralizing Abs can be also impaired by other serum Abs targeting the same antigen. To assess these aspects, we dissected the behavior of HSV positive human sera testing their binding to HSV glycoproteins, neutralization of infection and inhibition of cell-to-cell transmission. As expected, they all neutralized the infection, but none of them was able to inhibit cell-to-cell virus transmission nor inhibited mAb#33 activity when using them in combination with the mAb. Therefore,

Abs naturally elicited in humans are not always endowed with the important antiviral feature we described for mAb#33.

NEGLECTED ARBOVIRUSES IN HAITI: ARE WE MISSING THE ELEPHANT IN THE ROOM?

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In 2014 an outbreak of chikungunya virus (CHIKV) swept through the Americas causing nearly ~800,000 cases in 37 countries and raising a global alert toward a virus that was long considered a neglected tropical disease. The CHIKV outbreak was followed by a zika virus outbreak (ZIKV) that summoned a larger attention, with reports of microcephaly in pregnant women. The magnitude of both outbreaks resulted in a major shift in attention in research moving them from neglected to carefully studied arboviruses. It took more than fifty years for both viruses to catch the attention of the developed world to study them. How many future-causes of arbovirus outbreaks are being ignored today; an attempt to answer this question is done by University of Florida in Haiti, among a carefully studied cohort population of school children in a sub-urban area in the Oest Department. Plasma samples from children attending school clinic with symptoms of acute febrile illnesses are screened for arboviruses using molecular assays and virus culture. The later method resulted in identifying large number of viruses grouped in alphaviruses, flaviviruses and bunyaviruses occassionally co-infected with other blood borne pathogen. The data presented here displays the importance of using old school methods to detect arboviruses in tropical regions to prepare for the next global re-emerging outbreak and the imminent need for a global surveillance for neglected tropical arboviruses

HIGHLY PATHOGENIC H5N1- AND H5N8-TYPE AVIAN INFLUENZA A VIRUS (IAV) STRAINS: IMPACT OF POSSIBLE REASSORTMENT WITH CO-CIRCULATING EURASIAN H9N2-TYPE AVIAN IAV ON VIRAL REPLICATION AND PATHOGENICITY IN MAMMALS

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Humans are susceptible to infection with influenza A, B and C viruses. Influenza A viruses (IAV) represent worldwide circulating pathogens that cause annual epidemics and occasionally worldwide pandemics, infecting millions of people. In parallel, several high pathogenic avian influenza viruses (HPAIV) and low pathogenic avian influenza viruses (LPAIV) have (occasionally) been confirmed to cross the species barrier from birds to mammals/humans upon genetic reassortment (frequently with LPAIV/H9N2 strains) and/or antigenic drift. Since 1997, HPAIV/H5N1, HPAIV/H5N6, LPAIV/H6N1, LPAIV- and HPAIV/H7N9, LPAIV/H9N2, and LPAIV/H10N8 have successfully infected humans causing sporadic infections and/or fatalities. Increasing evidences that stable lineages of HPAIV/H5N1 or HPAIV/H5N8 and LPAIV/H9N2 viruses are being established in chickens and in different localities around the world especially Egypt and Germany, raise concerns about the possible reassortment between these highlighted strains. To early

recognize the impact of these possible reassortments on the mammalian and avian species, we discussed the impact of the genetic segments from intensively circulating LPAIV/H9N2 avian influenza viruses on the dynamic evolution of HPAIV/H5N1 and HPAIV/H5N8 reassortants with zoonotic potential in mammals or higher replication and pathogenicity in avian species.

GENISTEIN INHIBITS AFRICAN SWINE FEVER VIRUS REPLICATION IN VITRO BY DISRUPTING VIRAL DNA SYNTHESIS.

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African swine fever virus (ASFV) is the causal agent of a highly-contagious and fatal disease that affects domestic pigs. Once, neither an anti-viral drug nor effective vaccines selective are available; studies on new anti-ASFV molecules are urgently needed. Previously in our study (Hakobyan et al; 2016) it has been shown that compounds of plant origin have a very good prospective to become cheap and effective antiviral drug.

Here, we report that genistein hampers ASFV infection at non-cytotoxic concentrations, in Vero cells and porcine macrophages. Interestingly, the antiviral activity of this isoflavone, previously described as a topo II poison in eukaryotes and at higher concentrations, is maximal when is added to cells at middle-phase of infection (8 hpi), disrupting viral DNA replication (-99%), blocking the transcription of late viral genes as well as the synthesis of late viral proteins, reducing viral progeny. Further, the single cell electrophoresis analysis revealed the presence of fragmented ASFV genomes in cells exposed to genistein, suggesting that this molecule also acts as an ASFV-topo II poison and not as a reversible inhibitor. No antiviral effects were detected when genistein was added before or at entry phase of ASFV infection. Docking studies demonstrated that genistein may interact with four residues of the ATPbinding site of ASFV-topo II (Asn-144, Val-146, Gly-147 and Leu-148), showing more binding affinity (-4.62 kcal/mol) than ATP4-(-3.02 kcal/mol), emphasizing the idea that this viral enzyme has an essential role during viral genome replication and can be a good target for drug development against ASFV.

APPENDIX B: POSTER PRESENTATIONS FROM STUDENTS

THE PLANT ALKALOID BERBERINE INHIBITS HEPATITIS C VIRUS ENTRY BY TARGETING THE VIRAL E2 GLYCOPROTEIN

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Despite the advent of direct-acting antivirals (DAAs), HCV remains an important public health problem globally. There is currently no effective vaccine against the virus, and the DAAs in current use cannot prevent de novo infection, including in liver transplant setting wherein donor livers inevitably become re-infected. Developing entry inhibitors to HCV entry using nature-derived small molecules may help to expand/complement the current treatment options. Herein, using cell culture-derived HCV (HCVcc), viral pseudoparticles bearing HCV glycoproteins (HCVpp), and entry-related assays, we examined the effect of the plant alkaloid berberine (BBR) on HCV early viral entry. Our results show that BBR impeded HCVcc attachment and entry/ fusion steps without inactivating the free virus particles or affecting the expression of host cell entry factors. In addition, BBR also effectively inhibited HCVpp infection and molecular docking analysis pointed at potential interaction with HCV E2. Finally, BBR could suppress HCVcc infection of primary human hepatocytes. Our results, therefore, identify BBR as a potent HCV entry inhibitor, which merits further evaluation particularly for use in transplant setting.

BERBERINE AGAINST INFLUENZA - EFFECTIVE DRUG OR THE RESULT OF POORLY FITTED MODEL?

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Influenza A virus is unquestionably one of the most important human pathogens. According to WHO, it is estimated that yearly seasonal influenza causes 3 to 5 million severe cases and 300,000 to 650,000 deaths. High infectivity and variability force researchers to constantly seek new potential anti-influenza drugs. Berberine is an isoquinoline alkaloid found in many plants including *Berberis vulgaris* and has potent biological activity, i.e. anti-inflammatory, anticancer and antiviral properties. Studies also indicate its good anti-influenza properties. However, despite numerous reports on the activity, the mechanism of action against influenza virus is far from understanding, and the results are often incompatible or even contradictor. The aim of the research was to investigate the anti-influenza properties of berberine and to determine the mechanism of action. The experiments were carried out in a variety of cell lines susceptible to seasonal H3N2 influenza infection (*in vitro* model) as well as in human airway epithelium (HAE) cultures (*ex vivo* model prepare with primary cells). The CPE reduction assays, quantitative real-time PCR, and confocal microscopy were used for evaluation of the antiviral potential. Our results showed strong anti-influenza properties of berberine in HAE cultures and in human adenocarcinoma alveolar basal epithelial (A549) cells, while no inhibition was noted in the Madin-Darby Canine Kidney cells (MDCK). It is worth to note that MDCK cells constitute the most accepted *in vitro* model for the influenza infection. Further, we have noted that observed inhibition of virus replication in A549 cells is largely dependent on the cytotoxicity of berberine to tumor cells. Taken together, our results indicate that the effect of berberine is cell type-dependent and is based on indirect inhibition of influenza virus replication by modulation of intrinsic cellular pathways.

CELLULAR MODEL TESTING INHIBITORS OF ZIKA VIRUS NS3 PROTEASE.

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Zika virus is a flavivirus that has been known since 1947 but the first big outbreak was reported in the Federated State of Micronesia in 2007 whereas the association between Zika virus infection and brain disorders was demonstrated in 2015 in Brazil. Adults infected with this virus suffer mild symptoms such as fever, rash, headache and muscle pain although Guillain-Barré syndrome has also been reported. Infection is especially dangerous during pregnancy and can cause microcephaly and other neurological dysfunction in fetus. Zika virus encodes several proteins responsible for virus replication. Among these, the NS3 protein carries the protease activity, required for maturation of viral proteins, which is essential for virus replication. Due to above function scientist try to create substances that could possibly inhibit virus through the inhibition of its proteases. To study better protease activity we make an attempt to create a cellular model that will allow us test many different inhibitors. The inhibitors that show anti-protease activity will further be tested on Zika virus. Our model will be based on transfection of protease and specific substrate for protease in presence of inhibitors. In case of protease activity we observe signal inside cellular nuclei due to the attachment of nuclear localization signal to the substrate sequence. Using fluorescence microscopy we verify influence of inhibitors on protease.

FROM (Z)-4-(2-(2-(2-OXOINDOLIN-3-YLIDENE)HYDRAZINYL)THIAZOL-4-YL)ARYL TO 3'-ACETYL-5'-ARYL-1H-SPIRO[INDOLE-3,2'-[1,3,4]OXADIAZOLE]-2-ONE DERIVATIVES: STRUCTURAL EVOLUTION OF HIV-1 RT DUAL FUNCTION INHIBITORS.

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Pursuing on our investigation on isatin hybrid molecules as dual inhibitors of both associated functions of HIV-1 reverse transcriptase (RT), we identified diverse scaffolds such as 4-(2-(2-(2-oxoindolin-3-ylidene)hydrazinyl)thiazol-4-yl)aryl and -3-{2-[2-3-methyl-4-phenyl-2,3-dihydro-1,3-thiazol-2-ylidene]hydrazin-1-ylidene-2,3-dihydro-1H-indol-2-one derivatives. Both classes of compounds exhibited an interesting activity toward polymerase and ribonuclease functions. To further explore the chemical space and to increase the metabolic stability of the previously reported inhibitors we have designed and synthesised a new series of 3'-acetyl-5'-aryl-1H-spiro[indole-3,2'-[1,3,4]oxadiazole]-2-one derivatives. In these new derivatives we have substituted the potential Achilles' heel, constituted by the easily hydrolysable imine bond with a more stable spirane dihydro-oxadiazole spacer. Moreover, we have introduced a chiral centre that will be further investigated in order to evaluate the role of stereochemistry on the activity of the new series. Our preliminary results indicate that the racemic mixture of the new isatin-spirane hybrids is capable of inhibiting both RT functions.

HUMAN DDX3 PROTEIN: A NEW VALUABLE TARGET TO DEVELOP BROAD SPECTRUM ANTIVIRAL AGENTS

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Targeting a host factor essential for the replication of different viruses, but not for the cells, offers a higher genetic barrier to the development of resistance, may simplify therapy regimens for co-infections and facilitates management of emerging viral diseases. DDX3 is a human host factor required for the replication of several DNA and RNA viruses including some of the most challenging human pathogens currently circulating such as HIV-1, HCV, DENV and West Nile virus. DDX3 has multiple enzymatic activities (ATPase and RNA helicase) and functional domains that may be targeted by potential inhibitors. The first small molecules designed to inhibit the ATPase activity of DDX3 has been identified by our research group. However, the major drawbacks of such ATP-mimetics may be represented by the low selectivity for in vivo treatment. In a recent work, we designed and validated the first small molecule DDX3 inhibitors specifically targeting its RNA binding site. Pursuing this research line, a structure-based optimization process was prosecuted, resulting in the identification of a new family of more potent DDX3 inhibitors. We demonstrated for the first time that the inhibition of DDX3 by a small-molecule could be successfully exploited for the development of a broad spectrum antiviral agent. In addition to the multiple antiviral activities, hit compound retained full activity against drug resistant HIV-1 strains, in the absence of cellular toxicity. Pharmacokinetics and toxicity studies in rats confirmed a good safety profile and bioavailability of hit compound validating DDX3 as a novel important therapeutic target.

DUAL EFFECT OF THE MULTI-KINASE INHIBITOR MIDOSTAURIN ON ACUTE AND LATENT HIV-1 INFECTION

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In an effort to identify novel HIV-1 latency reversing agents (LRAs), we evaluated the effect of midostaurin in HIV-1 infection. Midostaurin is a multi-protein kinase inhibitor (including cyclin-dependent kinases, CDKs), that is used for the treatment of acute myeloid leukemia (AML). Midostaurin blocked HIV-1 replication in acutely infected cells at concentrations that were not cytotoxic. The antiviral effect was dependent on the expression of SAMHD1, a virus restriction factor which is, in turn, dependent on CDKs activity. Following SAMHD1 degradation expression the antiviral effect was lost and increased HIV-1 replication was observed as compared to untreated cells. In HIV-1 latently infected lymphoid cells, including clonal (ACH-2) and non-clonal models (J-Hig), midostaurin significantly increased HIV-1 reactivation. Moreover, a synergistic effect was observed when midostaurin was combined with known latency reversing agents (LRA), the HDAC inhibitors panobinostat and vorinostat, suggesting a distinct mechanism of action than the HDAC inhibitors.
In conclusion, we show a dual effect of midostaurin by blocking early steps of virus replication in acutely infected cells but promoting reactivation in latently infected cells. Our results suggest that agents such as midostaurin could effectively limit the size of the HIV-1 reservoir while preventing subsequent rounds of infection.

TISSUE-SPECIFIC RNA-SEQ REVEALS THE HERV CONTRIBUTION TO OUR TRANSCRIPTOME: PERSPECTIVES FOR UNCONVENTIONAL ANTIVIRAL STRA TEGIES

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Human Endogenous Retroviruses (HERV) are ancient proviral sequences integrated into the human genome, being four times more abundant than protein-coding genes. During the last 30 years, HERV expression has been intensively investigated due to its proposed role in many human diseases. Despite these efforts, a comprehensive analysis of HERV transcriptome and its genetic origin has not been performed yet, preventing the knowledge of the individual HERV loci expressed in physiological conditions and, by consequence, of their specific dysregulation in diseased tissues. Starting from a comprehensive dataset of about 3200 highly-conserved HERV loci, we analyzed public RNA-seq profiles of 26 tissues with a bioinformatics workflow developed ad hoc for the detection of HERV expressed products. We individuated a total of 189 coding-predicted RNAs deriving from intergenic HERV (ieHERVs) loci that are transcribed under physiological conditions. Among these, 17 transcripts were expressed at high levels and have been characterized in detail in terms of structure and predicted protein products. Overall, results offer a deep overview of the individual HERV loci accounting for a basal RNA expression in healthy conditions, providing hence a fundamental background to evaluate their specific dysregulation in pathological contexts. The HERV loci showing a differential expression in diseased tissue will constitute a valuable set of biomarker candidates, worth to be further investigated for their possible pathogenic contribution. Hence, the characterization of HERV transcriptome appears to be the way for the development of innovative and unconventional antiviral strategies, to counteract many non-infectious human illnesses with a poorly understood etiology.

EXTRACT OF *PARTHENIUM HYSTEROPHORUS* LEAVES CONTAINS ANTI-HIV-RT POTENTIAL

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An estimated 36.7 million people including children were living with the HIV till 2016 with a prevalence of 0.8% among adults. Natural products have been found to inhibit enzymes and proteins crucial to the life cycle of HIV. The toxicity of currently available anti-HIV drugs makes it difficult to maintain patient's adherence to antiretroviral therapy, as a consequence, the search for better anti-HIV agents continues and focused on natural sources, particularly the plant species. *Parthenium hysterophorus* L. (Asteraceae) also known as congress grass, is an annual herb. The *P. hysterophorus* leaves collected were shade-dried and grinded into fine powder with mortar and pestle. The powdered leaves were sequentially extracted using hexane (HX), benzene (BZ), chloroform (CH), ethyl acetate (EA), acetone (AC), ethyl alcohol (ET), and water (AQ) using Soxhlet apparatus. These extracts were centrifuged, filtered, and lyophilized. The dried residues were dissolved in DMSO. The reaction mixture consists of template/primer complex, dNTPs, and reverse transcriptase (RT) enzyme in lysis buffer with or without extract. After 1 h incubation at 370C, the reaction mixture was transferred to streptavidin coated microtiter plate (MTP). The biotin-labeled dNTPs that are incorporated in the template due to activity of RT were bound to streptavidin. The unbound dNTPs were washed and anti- digoxigenin-peroxide (anti-DIG-POD) was added to MTP. DIG- labeled dNTPs incorporated in the template were bound to anti- DIG-POD antibody. The unbound anti-DIG-POD was washed and peroxide substrate (ABTS) was added to MTP. A colored reaction product was produced during the cleavage of substrate catalyzed by peroxide enzyme. The absorbance of the sample was measured at 405 nm using microtiter plate ELISA reader. The resulting color intensity is directly proportional to the actual RT activity. The percentage inhibitory activity of extracts was calculated by comparing to a sample that does not contain an inhibitor. The *P. hysterophorus* extracts were evaluated for antiretroviral activity by targeting HIV-RT enzyme using HIV-RT kit (Roche). Anti-HIV- RT activity was measured at two different concentrations (0.6 and 6.0 ??g/ml), which showed low inhibition potential (<50%) *in vitro.* About 40% inhibition of reverse transcriptase (RT) activity was observed in hexane fraction at 6.0 ??g/ml concentration. The study showed that phytochemicals present in *P. hysterophorus* leaf have the potential agents with low to moderate anti-HIV-RT activity. The extracts (HX, ET, and AQ) produced modest anti-HIV-RT activity (about 23–40%). Nevirapine, the standard anti-HIV drug, showed 99.67% inhibitory activity. Since several phytochemicals are present in the crude extract, it might be possible that isolation and purification of the active ingredients from potential fractions of HX, ET, AQ and their bioactivity testing in the future may provide further enhancement of anti-HIV-RT activity.

MICROARRAY ANALYSIS OF GENOME-WIDE HOST RESPONSE TO INFECTION WITH DIFFERENT ISOLATES OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS (H5N1) IN DUCKS

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Ducks represent the classic reservoir for avian influenza with asymptomatic infection, recently some isolates of H5N1 highly pathogenic avian influenza viruses (HPAIV), have acquired the capacity for high pathogenicity in both domestic and wild ducks. Recent studies have added knowledge but still little is known concerning host/pathogen interactions during influenza infection in duck species. This study was carried to analyse and compare the genome-wide host response after infection with HPAIV H5N1 isolate 2008 (A/duck/Tripura/103597/2008 belonging to Clade 2.2) and isolate 2011 (A/duck/India/02CA10/2011 belonging to clade 2.3.2) in ducks utilizing microarray. A custom 60Kx8 microarray chip with duck specific probes containing total 22815 gene was hybridized with RNA extracted from duck lungs infected with HPAIV H5N1 isolate 2008 and isolate 2011. The gene expression profile of duck lung infected with HPAIV H5N1 isolate 2008 and isolate 2011 using GeneSpring GX 12.5 Software showed 1678 & 1186 significantly up-regulated and 1175 & 834 down-regulated genes respectively. 1074 genes were found commonly expressed between two HPAI H5N1 virus isolates. Genes exclusively expressed in HPAIV H5N1 isolate 2008 were approximately double in no. (1778) as compared to gene exclusively expressed in response to isolate 2011 (946). Commonly expressed genes between two HPAI H5N1 clades as well as genes which are expressed in response to infection with one HPAIV H5N1 isolate and were neutral in other were analysed with various intersections. The microarray data was annotated using Gene ontology analysis tool like High-Throughput GOMiner and DAVID. The various GO terms related to cytokine biosynthetic process, IFN-γ binding, apoptosis and inflammation related terms were found highly up-regulated in HPAIV H5N1 isolate 2011 as compared to isolate 2008. Important pro-inflammatory cytokine TGF-β related terms were enriched differently in response to HPAIV H5N1 clades 2.2 and 2.3.2. A probable hyper cytokine response and excess activation of innate immune response might be the causes of mortality in ducks in response to infection with HPAIV H5N1 isolate 2011 as compared to isolate 2008.

A SMALL MOLECULE THAT EFFICIENTLY INHIBITS DENGUE VIRUS ENTRY

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Dengue virus (DENV) poses a significant threat to inter-tropical region populations (~ half of global population), yielding 50 – 100 million annual infections. Specific preventive vaccines and treatments against the virus remain unavailable. Using lentivirus-based viral pseudoparticles bearing DENV glycoprotein E to screen for potential entry inhibitors against the flavivirus, herein we identified the compound PN1 that robustly inhibited DENV infection. PN1 could abrogate the infectivity of serotype 2 DENV and exhibited antiviral activity that particularly targeted DENV early viral entry, with minimal cytotoxicity and influence on other steps of the viral life cycle. Molecular docking analysis predicted potential interaction of PN1 with

domain II of the DENV glycoprotein E. Oral administration of PN1 in mice showed serum biodistribution and no apparent toxicity. Preliminary experiments demonstrated moderate protection against DENV-induced haemorrhage in animals treated with the small molecule. Altogether, these results support further exploration and development of PN1 as a potential candidate antiviral agent for the management of DENV infection.

PRODUCTION AND MOLECULAR CHARACTERIZATION OF RECOMBINANT PROTEINS ENCODED BY DENGUE VIRUS STRUCTURAL GENES OF LOCAL SEROTYPE-2

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Dengue virus (DENV) is a single-stranded positive-sense RNA virus belonging to the flavivirus family. DENV is an important infection which affects millions of people worldwide. Dengue Virus causes a complex disease ranging from dengue fever, to dengue haemorrhagic fever and dengue shock syndrome, which can be life threatening without medical intervention. For the early detection in the window period of dengue infection, the structural proteins including Core (C), Pre-membrane (preM) and Envelope (E) are using as the important diagnostic markers. Dengue structural proteins are also having important role for vaccine development as well as candidates for studying virus assembly and maturation. In the proposed study we will clone and express the structural genes from local Dengue virus serotype-2 in prokaryotic expression system and utilize the structural recombinant antigens to develop high-sensitive, specific and economical diagnostic assays for detection dengue infection, which could be utilized for serological screening. The purified recombinant antigens will be checked further immunogenically. The success of the proposed project will lead to develop in-house diagnostic assays, for the detection of dengue infection and to accelerate the development of new therapeutic vaccines for dengue infection.

STRUCTURE-BASED VIRTUAL SCREENING TO IDENTIFY SMALL-MOLECULE INHIBITORS OF DENGUE VIRUS TARGETING THE NS3-NS5 PROTEIN-PROTEIN INTERACTION

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Dengue virus (DENV) is a major insect-borne viral pathogen as it is responsible for almost 400 million new human infections annually, of which around 500,000 cases develop a severe disease with potentially lethal consequences. To date, no specific treatments or a fully protective vaccine are available against DENV. Therefore, there is an urgent need for the development of effective therapeutic strategies against DENV. NS3 and NS5 are the two most conserved DENV proteins and play a pivotal role in the viral life cycle. The NS3-NS5 interaction is essential for DENV replication complex and disrupting this interaction could represent an attractive approach for the design of broad-spectrum anti-DENV inhibitors. Starting from the available crystallographic structures of NS3 and NS5 DENV proteins, we identified a potential druggable cavity at the NS3/NS5 interface. This cavity was used as a template for a structure-based virtual screening of a database consisting of over 3 million commercially available small molecules to search for potential inhibitors of this interaction. After that process, top-ranked compounds were selected and will be subjected to biological characterization. Taken together, this study proposes an innovative and multidisciplinary antiviral approach against DENV that may be applied to other protein-protein interactions necessary for the viral life cycle.

A-TO-I EDITING BY ADAR1 LIMITS HPV EXPRESSION BY REGULATING INNATE IMMUNITY

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Infection by human papillomavirus (HPV) alters the microenvironment of keratinocytes as a mechanism to evade the immune system. A putative therapeutic strategy against HPV infection would be the induction of an anti-inflammatory state to avoid immune response. Therefore, understanding the role and function of innate immune effectors and modulators can help to establish novel strategies for HPV treatment. A-to-I editing by ADAR1 has been reported as a key step in triggering innate immunity in response to foreign viral RNAs.
We used human keratinocyte HaCaT(HPV-) and SiHa(HPV16+) cell lines to characterize innate immune activation in the context of HPV infection. SiHa HPV16+ cells showed lower expression of ADAR1, and higher expression of RIG-I and phosphorylated STAT1 compared to HaCaT HPV- cells. Thus, RNAi was used to specifically downregulate *ADAR1* for further characterization. *ADAR1* knockdown (siADAR1) in HPV16+ cells induced increased expression of *IFIH1*/MDA5 (24-fold), *DDX58*/RIG-I (20-fold), *IRF7* (13-fold), *IFNB1* (100-fold) and *CXCL10* (490-fold) compared to mock-transfected cells. Importantly, siADAR1 significantly enhanced *HPV16* expression, indicating ADAR1 and its downstream effectors may modulate HPV infection by innate immune activation. Functionality of ADAR1 and its ability to edit HPV transcripts was evaluated. A-to-I editing was found in known cellular target of ADAR1 (*NEIL1*), but was not identified in HPV transcripts, suggesting the effect of ADAR1 on HPV is editing-independent. In summary, we demonstrate that ADAR1 and its downstream effectors have an antiviral role in HPV infection through the induction of innate immunity. Hence, targeting ADAR1 could be a strategy against HPV infection and disease.

DEVELOPMENT OF NOVEL 1,3,5-TRIAZINES TARGETING THE ENTRANCE CHANNEL OF THE HIV-1 NNRTI BINDING POCKET

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Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are vital component of highly active antiretroviral therapy (HAART) for the treatment of HIV-1 infection. Nevertheless, these agents also facing the crisis of drug-resistance and severe side-effects which puts a selective pressure to develop novel NNRTIs with potent pharmacological profile with less side effects. The clinical utility of currently available NNRTIs has been also compromised seriously owing to unpleasant aqueous solubility. Encouraged by the above, present project attempts to develop novel aqueous soluble hybrid derivatives of 1,3,5-triazine with established anti-HIV pharmacophores (such as phenylthiazole, thiazolidine-2,4-dione, thizolidinone, and oxadiazoles) by maintaining morpholine to improve aqueous solubility with possible engagement of entrance channel of HIV-1 RT. These triazine molecules were able to internalize in the largely open region (entrance channel) in front of Lys103, Glu138, and Val179 other than the conventional tunnel and groove lined by Tyr181, Tyr188, Phe227, Trp229, Tyr318, Pro225, and Pro236 in Non-Nucleoside Inhibitor Binding Pocket (NNIBP)of HIV-1 RT. This new region still has been less explored in the development of NNRTIs and worth to be investigated.

STRUCTURE- BASED IDENTIFICATION OF PEPTIDOMIMETIC INHIBITORS OF CHIKUNGUNYA VIRUS AND DETERMINATION OF THEIR ANTIVIRAL ACTIVITY.

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Chikungunya (CHIKV), an arbovirus is responsible for causing fever and arthritis in humans. Currently, no vaccine or antiviral are available against CHIKV infection. CHIKV nsP2pro, a critical viral enzyme rocess the

nonstructural polyprotein into individual nsPs for the formation of viral replication-complex and thus constitutes a promising drug target. In this study, crystal structure of nsP2pro is elucidated and structure-based protease inhibitors are identified. An eight aa long substrate oligopeptide corresponding to nsP3/4 cleavage site was docked in the active site of nsP2pro which reveals the molecular determinants and critical interaction of enzymatic active site. Further, a series of peptidomimetic inhibitors was identified by mimicking the critical interactions of the substrate peptide nsP3/4 in the active site of nsP2pro. Identified compounds were then analyzed by docking for their structural stability and conformational flexibility and their binding affinity was assessed by molecular dynamic simulations. Two of the peptidomimetic compounds were further screened by a FRET based assay to examine the inhibition of CHIKV nsP2pro. Both the compound Pep-I & Pep-II inhibited nsP2pro activity with IC50 values of 34μM and 42μM, respectively. The inhibition kinetic studies showed that the inhibition constant (Ki) is 33.34±2.53 μM for Pep-I and 45.89±4.38 μM for Pep-II. Additionally, these two compound were further validated by plaque reduction assay and found to be significantly inhibiting CHIKV replication in BHK-21 cells. This approach of structure-based identification and validation of peptidomimetic compound to inhibit CHIKV nsP2pro pave the way to design novel antiviral drugs against chikungunya infection.

A VP1 MUTATION ACQUIRED DURING AN ENTEROVIRUS 71 DISSEMINATED INFECTION CONFERS HEPARAN SULFATE BINDING ABILITY AND MODULATES *EX VIVO* TROPISM.

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Enterovirus 71 (EV71) causes hand, foot and mouth disease, a mild and self-limited illness that is sometimes associated with severe neurological complications. EV71 neurotropic determinants remain ill-defined to date. We previously identified a mutation in the VP1 capsid protein (L97R) that was acquired over the course of a disseminated infection in an immunocompromised host. The mutation was absent in the respiratory tract but was present in the gut (as a mixed population) and in blood and cerebrospinal fluid (as a dominant species). In this study, we demonstrated that this mutation does not alter the dependence of EV71 on the human scavenger receptor class B2 (SCARB2), while it enables the virus to bind to the heparan sulfate (HS) attachment receptor and modifies viral tropism in cell lines and in respiratory, intestinal and neural tissues. Variants with $VP1_{97L}$ or $VP1_{97R}$ were able to replicate to high levels in intestinal and neural tissues and, to a lesser extent, in respiratory tissues, but their preferred entry site (from the luminal or basal tissue side) differed in respiratory and intestinal tissues and correlated with HS expression levels. These data account for the viral populations sequenced from the patient's respiratory and intestinal samples and suggest that improved dissemination, resulting from an acquired ability to bind HS, rather than specific neurotropism determinants, enabled the virus to reach and infect the central nervous system. Finally, we showed that iota-carrageenan, a highly sulfated polysaccharide, efficiently blocks the replication of HS-dependent variants in cells and 2D neural cultures. Overall, the results of this study emphasize the importance of HS binding in EV71 pathogenesis and open new avenues for the development of antiviral molecules that may prevent this virus's dissemination.

PRODUCTION OF CONSERVED ANTIGENIC RECOMBINANT E6 ONCOPROTEIN FROM HIGH RISK HUMAN PAPILLOMAVIRUS IN 293 CELL LINE

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Human papillomavirus (HPV) induced cervical cancer is the fourth most common cancer and the second most common cause of death in females, globally. To date three prophylactic vaccines i.e. Gardacil 4 and

Gardacil 9 by Merck and Cervarix by Glaxo Smithkline have been confirmed to prevent high risk HPV (HR-HPV) types. Unlike other developing countries, Pakistan has no data on vaccination against HPV. Since prophylactic vaccines are preventive only, most researches are shifted towards therapeutic vaccine development that could prevent and treat as well. Two regulatory proteins E6 and E7 are the efficient targets for therapeutic vaccines development. We proposed to isolate locally prevalent HR-HPV types from cervical cancer tissues. After amplification and sequencing, the most conserved antigenic region from E6 genes of different HR-HPV types will be predicted and cloned in a ubiquitous chromatin opening elements (UCOE) containing lentiviral vectors, specifically designed for the stable transfection in 293 cell line. Using this optimized system, a high yield of correctly folded and appropriately post-translationally modified recombinant E6 protein will be obtained that is supposed to be highly immunogenic. This protein could be used for further therapeutic functional analysis.

A SINGLE-CHAIN ANTIBODY SPECIFIC FOR THE E6 ONCOPROTEIN OF HUMAN PAPILLOMAVIRUS TYPE 16 AS A THERAPEUTIC TOOL FOR THE VIRUS-ASSOCIATED LESIONS

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Worldwide, approximately 600000 cases/year of high-risk HPV-related tumors occur, where cervical cancer (CC) is predominant and represents the fourth most common cancer in women, and the oropharynx cancers are constantly increasing, representing a serious problem of public health. The HPV16 is the prevalent genotype in all HPV-tumors. The HPV-diseases can be prevented through vaccination but other strategies are needed to prevent neoplastic progression in already infected people. HPV oncogenicity depends on the E6 and E7 viral proteins, which are constitutively expressed only in tumor cells, where alter homeostasis by interacting with several cellular proteins. Thus, E6 and E7 represent ideal therapeutic targets. In recent years, recombinant antibodies in single-chain format (scFvs) expressed as intrabodies were used to hamper the functions of specific targets. We previously reported that an intrabody (scFvI7) specific for the HPV16-E6 inhibited proliferation of HPV16+ cells in vitro and reduced tumor development in HPV-cancer animal models. To develop therapeutic tools as safe as possible for use in humans, the scFvI7 was obtained in protein format by expression and purification in E. Coli. The specificity and sensitivity for E6 of the scFvI7 protein with and without signal for nuclear localization (NLS) were characterized by immunoassays in vitro, and the ability to recognize the endogenous E6 in HPV16+ cells was confirmed by Immunofluorescence and Confocal microscopy. The stability of the scFvI7 proteins in physiological conditions and their efficacy to hamper viability and proliferation of HPV16+ cells were also analysed in vitro as essential features for future in vivo applications.

IDENTIFICATION OF NOVEL INHIBITORY AGENT TO HUMAN IMMUNODEFICIENCY VIRUS (HIV) ENTRY USING HIV-ENV PSEUDOTYPED VIRUS

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At present, the AIDS-inducing HIV remains a significant medical burden worldwide, with over 37 million carriers and about 2 million new infections yearly. Although non-curative, antiretroviral (ARV) drugs have achieved considerable success in reducing HIV-associated morbidity and mortality, continuous efforts in developing additional antivirals, such as entry inhibitors, are essential to enhance the present day management of the HIV epidemic. Viral pseudoparticle systems bearing HIV Env (HIVpp) can be used to mimic the early viral entry steps and are particularly useful for the discovery of viral entry inhibitors. In this study, we employed a reporter-based HIVpp and identified a candidate HIV entry inhibitor from a natural product library. Results revealed that the compound NP-1 inhibited HIVpp infection of Jurkat cells by >80% at non-cytotoxic doses and the antiviral activity was dose-dependent. Kinetic analysis of drug treatment over time on infection by GFP-marked NL4.3 WT virus revealed that NP-1 severely impaired the production of infectious HIV and suppressed the number of virus-induced GFP-positive Jurkat cells. Further mechanistic studies of NP-1's effect against distinct steps of the HIVpp entry and other stages of the viral life cycle are underway. Our results also demonstrate the utility of the HIVpp system in discovering novel entry inhibitors to HIV, which has profound impact to help expand the scope of candidate HIV antivirals for prophylactic and/or therapeutic application.

INVESTIGATION OF THIENOPYRIMIDINONE DERIVATIVES AS DUAL INHIBITORS TARGETING THE HIV-1 REVERSE TRANSCRIPTASE-ASSOCIATED RIBONUCLEASE H FUNCTION AND INTEGRASE ACTIVITY.

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Despite the approval drugs for Human Immunodeficiency Virus type 1 (HIV-1) therapy, emergence of drug resistance strains and side effects due to chronic drug administration require the identification of new HIV-1 inhibitor. In this work, we investigate the effects of thienopyrimidinone derivatives on both Reverse Transcriptase (RT)-associated Ribonuclease H (RNase H) activities and HIV-1 Integrase (IN) in biochemical assays. In the quest of developing novel and selective HIV-1 RNase-H allosteric inhibitors, we observed that some thienopyrimidinone derivatives, although more active on RT, also inhibited IN LEDGF-dependent activity. A key role of the poly-hydroxylated aromatic residue linked to the pyrimidinone scaffold was noted. In particular, we observed that that simple introduction of an OH group in 2 position of the catechol moiety of GZ 510 (a highly selective and efficient RNase inhibitor), invoked a dramatic improvement in IN activity inhibition. In fact, the compound GZ 675, while maintaining RNase inhibition, is 55 fold more potent that GZ510 in inhibiting HIV integrase, with an IC50= 0.45 micromol. Intrigued by this interesting result, in the study presented herein, we will report on the significance of the number and of the position of the hydroxyl groups. The role of the hydrophobic residue on the thieno ring was also explored, representing a good scaffold for dual HIV inhibitors for further developments.

APPENDIX C: FINANCIAL SUPPORT

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