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Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

ROME TECHNOPOLE
INNOVATION ECOSYSTEM

7th IAAASS
Pula (CA) 23-27/09/2024
Innovative Approaches for identification of Antiviral Agents
Summer School

7th Innovative Approaches for Identification of Antiviral Agents Summer School

September 23th-27th 2024,
Santa Margherita di Pula, Sardinia, Italy

Program & Abstract Book

UNICA
UNIVERSITÀ DEGLI STUDI DI CAGLIARI

SARDIGNA CHIRCAS
SARDEGNA RICERCHE

Dear Participant,

Following the success of the previous editions, we have the pleasure to welcome you to the "Innovative approaches for the identification of antiviral agents" summer school, organized by the Department of Life and Environmental Sciences of the University of Cagliari and Sardegna Ricerche in the frame of Next Generation Virology initiative and the Antiviral DiscoVery Initiatives: Educating Next-Gen Scientists (acronimo ADVISE) with the patronage of Regione Autonoma della Sardegna, European Society for Virology, Federation of European Microbiological Societies, Italian Society for Microbiology and Molecular Biology and International Antiviral Symposium Foundation.

Over the recent years, drug discovery via high throughput screening (HTS) has moved beyond the boundaries of pharmaceutical companies and been successfully integrated into research programs of many academic institutes. These efforts have taken advantage of the availability of diverse libraries of small molecules, either as pure entities or natural product extracts. However, identification of candidate drugs benefits from an in-depth understanding of potential pitfalls of HTS, which can include (i), storage of compound libraries (ii), stability and cost of assay reagents (iii), robustness of the assay and (iv), access to bioinformatics to analyze the wealth of data that results from performing multiple assays on 250,000 – 500,000 compounds. Beyond these challenges, successful HTS is an iterative process requiring close co-operation with programs of structural biology, medicinal chemistry and clinical research.

With these issues in mind, the Innovative Approaches for Identification of Antiviral Agents Summer School (IAAASS) aims to provide an informal and interactive environment to review the application of HTS techniques to the identification of novel and clinically-significant antiviral drugs. The Summer School is targeted to researchers at an early stage in their career, combining examples of drug discovery from internationally-recognized experts in the field with informal small-group thematic discussion sessions.

The Organizing Committee welcomes you to the Sardegna Ricerche Research Park and to the Hotel Flamingo, Santa Margherita di Pula, located on the south tip of Sardinia and looks forward to sharing with you their experience on current and future strategies for identifying novel antiviral agents targeted to clinically-significant diseases.

The Summer School Organizing Committee

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ROME TECHNOPOLE INNOVATION ECOSYSTEM

Program

Program

2024.09.23 - Monday		
15:30	Shuttle from Hotel Flamingo to the Research Park	
16:30	**Opening Remarks** *Chair Enzo Tramontano*	***Auditorium***
	Plenary Lectures	
17:00-17:45	Broader-acting antivirals for epidemic and pandemic preparedness	**Johan Neyts** KU Leuven
17:45-18:30	A Biochemist's Journey of Antiviral Drug Discovery	**Joy Feng** San Francisco
18:30	Shuttle from Research Park to the Hotel Flamingo	
20:00	**Dinner**	

2024.09.24 - Tuesday		
8:15	Shuttle from Hotel Flamingo to the Research Park	
	Plenary Lecture *Chair Reuben Harris*	***Auditorium***
9:00-9:45	Using DNA-Encoded Library Screening to Effectively and Rapidly Develop New Antiviral Drugs	**Damian Young** BCM Houston
9:45-11:00	**Selected talks – session I O.1-O.6**	
O.1	Design and Synthesis of Novel Macrocycle Inhibitors Targeting SARS-CoV-2 Main Protease	**Alessia Alberico** UNINA
O.2	Studies on the SARS-CoV-2 Mpro dimerization mechanism using a split luciferase assay	**Renee Delgado** UT Health
O.3	Discovery of novel non-nucleoside inhibitors against SARS-CoV-2 minimal replication-transcription complex	**Paolo Malune** UNICA
O.4	The in vitro genetic barrier to resistance of lenacapavir is not affected by viral subtype or heavy treatment exposure	**Chiara Paletti** UNISI
O.5	New strategies for inhibiting Hepatitis B Virus entry using Cyclosporine A analogues	**Marika Longo Minnolo** UNIMI
O.6	Exploring thiazole systems for antiviral applications: synthesis and biological evaluation	**Roberta Bivacqua** UNIPA
11:00	**Coffee Break**	
	Plenary Lecture *Chair Johan Neyts*	***Auditorium***
11:30-12:15	Challenges and opportunities in the management of antiviral drug resistance among DNA viruses	**Graciela Andrei** KU Leuven
12:15-13:15	**Poster session I - odd numbers**	***Workshop Room***
13:30-14:30	**Lunch**	

2024.09.24 - Tuesday		
	Plenary Lecture *Chair Graciela Andrei*	***Auditorium***
14:30-15:15	First in Class and Best in Class in antiviral drug discovery: two different meaning, challenges and opportunities	**Vincenzo Summa** UNINA
15:15-16:30	**Selected talks – session II 0.7_O.12**	
O.7	Investigating the role of lake microbial enzymes on virus inactivation	**Daniela Morales Duran** EPFL
O.8	Targeting HSV-1 through FASN inhibitors: implications for Alzheimer's Disease	**Linda Trifirò** UNITO
O.9	Novel Fusion Inhibitors Targeting Hemagglutinin (HA) of Influenza A H1N1 Virus	**Álvaro de la Cruz** CSIC IMQ
O.10	Fusion inhibitory peptides inhibit SARS-CoV-2 Omicron BA.5 transmission in hamsters	**Kim J. Handrejk** Erasmus MC
O.11	Elucidation of the pan-flavivirus potential of NS4B-targeting antivirals	**Dominik Kiemel** CIID
O.12	Exploiting the potency of new DKA derivatives as SARS-CoV-2 nsp13 inhibitors and their role in blocking viral replication	**Roberta Emmolo** UNICA
16.45	Shuttle from Research Park to the Hotel Flamingo	
18:30	**Discussion Groups**	
20:00	**Dinner**	

2024.09.25 - Wednesday		
8:15	Shuttle from Hotel Flamingo to the Research Park	
	Plenary Lectures *Chair Peter Holst*	***Auditorium***
9:00-9:45	Outflanking the Pathogenic Arenaviruses	**Brian Gowen** Logan USU
9:45-10:30	HIV: to integrate, or not to integrate, that is the question	**Ben Berkhout** Amsterdam UMC
10:30-11:00	**Selected talks – session III O.13-O.14**	
O.13	Polyoxometalate exerts broad-spectrum activity against human respiratory viruses hampering viral entry	**Elisa Feyles** UNITO
O.14	A novel class of SARS-COV-2 nsp13 Helicase and NTPase inhibitors: Pyridobenzothiazolone (PBTZ)-based compounds	**Anastasia Ferraro** UNINA
11:00	**Coffee Break**	
	Plenary Lecture *Chair Valeria Cagno*	***Auditorium***
11:30-12:15	Exploiting Ebola virus suppression of the innate immune activation as target for drug development	**Angela Corona** UNICA
12:15-13:15	**Poster session II - even numbers**	***Workshop Room***
13.30-14.30	**Lunch**	

2024.09.25 - Wednesday		
	Plenary Lecture *Chair Elias Maccioni*	***Auditorium***
14:30-15:15	Flex-Nucleosides – a strategic approach to broad-spectrum antiviral therapeutics	**Kathie Seley-Radtke** UMBC Baltimore
15:15-16:30	**Selected talks – session IV 5 O.15- O.20**	
O.15	Amphibian peptide HS-1 and its ala-Scanning analogues as broad-spectrum antivirals	**Bianca Maria Nastri** UNICM
O.16	Multi-Omics Profiling to Unveil West Nile Virus interactions with Infected cells	**Sante Scognamiglio** UNICA
O.17	Discovery of thioaryl imides as a new family of SARS-CoV-2 entry inhibitors	**Víctor Fernández Cabello** CSIC
O.18	Molecular evolution of the hepatitis B virus in the presence of some promising antiviral plant extract formulations	**Giscard W. Koyaweda** Paul-Ehrlich-Institut
O.19	Prevention of lethal SARS-CoV-2 replication in human ACE2-transgenic mice through error prone suggests that daclatasvir dose can be adjusted for early COVID-19 therapy	**Mayara Mattos** IOC Fiocruz/Brazil
O.20	Evaluation of broad-spectrum piperazine-based compounds able to inhibit flavivirus and/or SARS-CoV-2 replication in a live virus assay	**Ilenia Varasi** UNISI
16.30	Shuttle from Research Park to the Hotel Flamingo	
18:30	**Discussion Groups**	
20:00	**Dinner**	

2024.09.26 - Thursday		
8:15	Shuttle from Hotel Flamingo to the Research Park	
Public/private synergies in viral research *Chair Enzo Tramontano*		***Auditorium***
	Plenary Lectures – Biotech companies	***Auditorium***
9:00-9:45	Development of immunotherapy based on genetically delivered Human endogenous retrovirus-K based VLPs	**Peter J. Holst** Hervolution
9:45-10:30	Data informed drug discovery, software tools and case studies	**Philip Gribbon** Fraunhofer Inst.
10:30	**Coffee Break**	
11:00-12:30	***Round table "Is there life after the PhD?"*** *Chair Vincenzo Summa* **Philip Gribbon** Fraunhofer Inst. **Peter J. Holst** Hervolution ***Emanuela D'Acunto*** *Takis Biotech* ***Valeria Cagno*** *CHUV Lovanio*	***Auditorium***
12.30-14:00	**Lunch**	
Public/private synergies in viral research		***Auditorium***
14:00-14:30	**Roberto Anedda** (Porto Conte Ricerche): NMR in antiviral drug discovery: the project of the NMR Platform in Sardinia	
14:30-14:50	**Ilaria Trudu** (Sardegna Ricerche): International opportunities for research and innovation, the role of the Enterprise Europe Network (EEN Network)	
14:50-15:10	**Akash Deep Bisvas** (Dompè Pharmaceuticals): The LIGATE Project: Harnessing AI for Advanced Drug Discovery Against Viral Infections	
15:10-15:40	**Emanuela D'Acunto** (Takis Biotech) :Isolation and Characterization of Neutralizing Monoclonal Antibodies from a Large Panel of Murine Antibodies against RBD of the SARS-CoV-2 Spike Protein	
15:40-16:10	**Franco Lori** (Virostatics): Revamping Sanitation: How SARS-CoV-2 Has Elevated the Role of Biocides in Closed Environments	

2024.09.26 - Thursday		
Public/private synergies in viral research *Chair Enzo Tramontano*		***Auditorium***
Plenary Lectures – Biotech companies		***Auditorium***
16:10	***Round table «Opportunities for Regional development of Biotech research »*** **Peter J. Holst** Hervolution **Philip Gribbon** Fraunhofer Inst. **Emanuela D'Acunto** Takis Biotech **Akash Deep Bisvas** Dompè Pharmaceuticals **Marco Naseddu** Regione Sardegna (TBC **Vincenzo Summa** UNINA**Franco Lori** ViroStatics	
17:30	Shuttle from Research Park to the Hotel Flamingo	
20:00	**Dinner**	

2024.09.27 - Friday		
8:15	Shuttle from Hotel Flamingo to the Research Park	
Plenary Lectures *Chair Kathie Seley-Radtke*		***Auditorium***
9:00-9:45	Innovative strategies for antiviral development: from viral RNA to attachment mimicry	**Valeria Cagno** CHUV Lausanne
9:45-10:30	Anthranilic Acid based Dihydroorotate Dehydrogenase Inhibitors for the Use as Antiviral Agents	**Chris Meier** Hamburg University
10:30	**Coffee Break**	
Plenary Lecture *Chair Ben Berkhout*		***Auditorium***
10:30-12:15	Cellular APOBEC3 enzymes and herpesviruses – why we should care!	**Reuben Harris** UT Health San Antonio
12:15-13:15	**Poster session III- All**	***Workshop Room***
13:15-14.30	**Lunch**	
Plenary Lecture *Chair Angela Corona*		***Auditorium***
14:30-15:15	Tools and Tactics for Targeting RNA with Small Molecules	**Jay Schneekloth** NIH Frederick
15:15-16:10	**Selected talks – session V O.21 – O.24**	
O.21	AMALPHI: A Machine Learning Platform for Predicting Drug-Induced Phospholipidosis	**Maria Cristina Lomuscio** UNIBA
O.22	Plant-derived Antiviral Strategies: Antiherpetic Properties of Tomato Root Exudate and Antimicrobial Efficacy of Soybean-Derived plasticizer	**Greta Bajetto** UNIPO
O.23	Structural and Functional Characterization of Receptor Binding Domain (RBD) for the reconstructed HERV Envelopes and their interaction with Host Cellular Receptors	**Saili Chabukswar** UNICA
O.24	Anchimerically Activatable Prodrugs of Remdesivir Nucleotide Monophosphate with Enhanced Metabolic Stability for Oral Delivery	**Jacob A. Smith** UMN Minneapolis
16:15	Shuttle from Research Park to the Hotel Flamingo	
18:00	**Not only science: traditional volleyball match!**	
20:00	**Closing dinner**	

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7th IAAASS

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Innovative Approaches for identification of Antiviral Agents Summer School

Poster List

Poster number	Author		Affiliaton	Title
P1	Artur	Akhremchuk	Institute of Microbiology of the National Academy of Sciences of Belarus. Kuprevich str. 2, 220084, Minsk, Belarus	**Viral Communities in Antarctic lake core samples**
P2	Aroa	Arboleya Agudo	University Institute of Biotechnology of Asturias (IUBA). Department of Biochemistry and Molecular Biology. University of Oviedo, Oviedo, 33006, Spain	**RHDV–S domain based subviral particle formation**
P3	Giulia	Atzeni	Department of Life and Environmental Sciences, University of Cagliari ,Cittadella Universitaria di Monserrato, S.P. 8 km 0.700, 09042, Cagliari	**Unveiling new scaffolds for the inhibition of West Nile NS3 helicase**
P4	Elena	Bianchi	Institute of Molecular Genetics, IGM-CNR "Luigi Luca Cavalli-Sforza", Via Abbiategrasso 207, 27100 Pavia, Italy	**Exploring the broad-spectrum antiviral potential of Bithiazole derivatives targeting PI4KB**
P5	Noemi	Cabella	Institute of Molecular Genetics IGM-CNR "Luigi Luca Cavalli-Sforza", National Research Council, via Abbiategrasso 207, Pavia, Italy	**Development of a virus-free cell assay for the evaluation of viral protease inhibitors**
P6	Claudia	Cabiddu	Università degli Studi di Cagliari; Dipartimento di Scienze della Vita e dell'Ambiente, Laboratorio di Virologia molecolare	**Identification of specific HERV loci differentially expressed in Multiple Sclerosis patients as potential biomarkers and therapeutic targets.**
P7	Ignasi	Calba	Institut the research IrsiCaixa. Hospital Germans Trias i Pujol, Barcelona	**harnessing the innate immune response through Nod1 agonists prevents SARS-CoV-2 infection in human lung epithelial cells**
P8	Marianna	Camasta	Department of Life and Environmental Sciences, University of Cagliari, 09042 Monserrato (CA) SS554, Italy	**Characterisation of the mechanisms of interferon production inhibition by Ebola virus VP35 wild-type and mutants**
P9	Marta Maria	Cara	Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato	**The expression of Human Endogenous Retroviruses in PBMC is modulated by SARS-CoV-2 acute infection and shows a specific transcriptional pattern as compared to other COVID-19 clinical stages**
P10	Gianfranco	Cavallaro	Università degli Studi di Catania - Dipartimento di Scienze Chimiche - Supervisor Professor Cosimo Gianluca Fortuna	**Diarylheterocycle: in silico design throught Molecoular Modelling studies of a new potentially inhibitors active against main protease of SARS-CoV-2.**
P11	Hanna	Daineka	Institute of Microbiology of the National Academy of Sciences of Belarus; Kuprevicha street, 2, Minsk, Belarus	**Recombinant nucleoside-2´-deoxyribosyltransferase Lactobacillus delbrueckii application for green nucleoside analogs production**
P12	Laura	Demuru	Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato	**Exploring the structural features for the identification of ZIKV NS3pro allosteric inhibitors**
P13	Laura	Dettori	Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato	**2-phenylquinoline derivatives activity on SARS-CoV-2 nsp13 helicase: insights from enzymatic and cell-based assays**
P14	Martin	Ferrié	KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, 3000 Leuven, Belgium.	**Mapping the druggable antiviral targets in the replication cycle of mammarenaviruses**
P15	Benedetta	Giuliani	Laboratory of Microbiology and Virology, Vita-Salute San Raffaele University, Milan, Italy	**Proper Selection of In Vitro Cell Model Affects the Characterization of the Antiviral Neutralizing Antibody Response**
P16	Yana	Kananovich	Institute of Microbiology, National Academy of Sciences, Kuprevich str. 2, 220084, Minsk, Belarus	**Enzymatic Preparation of Phospholipid Derivatives of Entecavir**
P17	Yuxia	Lin	KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, 3000 Leuven, Belgium.	**A Robust Mouse Model of Human Parainfluenza 3 Virus Infection to Study Prophylactic and Therapeutic Modalities**
P18	Camilla	Lodola	Institute of Molecular Genetics IGM CNR "Luigi Luca Cavalli-Sforza", Via Abbiategrasso 207, 27100 Pavia, PV, Italy	**Modulation of SARS-CoV-2 Nucleocapsid RNA binding by the host RNA helicase DDX3X**

Poster number	Author		Affiliaton	Title
P19	Miguel	Maldonado-Menéndez	Instituto de Química Médica (IQM-CSIC), Juan de la Cierva 3, Madrid, Spain	**Triazole-Based Compounds as Efficient Dimerization Disruptors of Leishmania infantum Trypanothione Reductase**
P20	Stefania	Maloccu	Università degli Studi di Cagliari; Dipartimento di Scienze della Vita e dell'Ambiente, Laboratorio di Virologia molecolare	**Development of a miniaturized high-performance FRET-based assay for screening of Nsp15 inhibitors**
P21	Federica	Mastrolembo Barnà	Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale SS. Annunziata, 98168 Messina, Italy	**Antiviral properties exerted by Pistachio extracts and zeaxanthin against SARS-CoV-2 variants in vitro binding models**
P22	Josephine	Meibom	EPFL-ENAC-IIE-LEV, GR A0 382 (Bâtiment GR), Station 2, 1005 Lausanne, Switzerland	**Determining the proteolytic fingerprint of Lake Geneva to investigate the mechanism of virus inactivation in lake water**
P23	Miroslav	Metodiev	Stephan Angeloff Institute of Microbiology, 26 Georgi Bonchev street 1113, Sofia, Bulgaria	**in vitro cytotoxicity and anti-influenza activity of silver nanoparticles loaded in various polymer drug delivery systems for topical administration**
P24	Areli Jahzeel Andrea	Navarro Lazcano	Pontificia Universidad Catolica de Chile, Portugal 47, Santiago, Chile	**Role of cyclooxygenases 1 and 2 in herpes simplex type 1-infected dendritic cells**
P25	Salvatore	Nieddu	Università degli Studi di Cagliari; Dipartimento di Scienze della Vita e dell'Ambiente, Laboratorio di Virologia molecolare	**Optimization of high-sensitivity assay for HTS of potential inhibitors against WNV NS2B-NS3 protease.**
P26	Alessia	Onali	Università degli Studi di Cagliari - Dipartimento di Scienze della Vita e dell'Ambiente	**Identification of new viral helicase inhibitors via integrated computational and synthetic approaches**
P27	Maria Michela	Pallotta	Institute of Molecular Genetics, IGM CNR "Luigi Luca Cavalli-Sforza", Via Abbiategrasso 207, 27100 Pavia, Italy	**DDX5 an emerging treater for SARS-CoV-2 infection**
P28	Blanca	Palmero Casanova	University of Castilla-La Mancha	**Repurposing AKt inhibitors for the treatment of Orthoflavivirus infection**
P29	Annalaura	Paulis	Università degli Studi di Cagliari; Dipartimento di Scienze della Vita e dell'Ambiente, Laboratorio di Virologia molecolare	**Development of hybrid hydroxychloroquine liposomes with Ag-nanoparticles as pan-HCoVs antivirals.**
P30	Sara	Piras	Università degli Studi di Cagliari; Dipartimento di Scienze della Vita e dell'Ambiente, Laboratorio di Virologia molecolare	**Differential expression of Human Endogenous Retroviruses in Chronic and Acute Myeloid Leukemia at Diagnosis and after TKI Therapy**
P31	Ekaterina	Ryabchevskaya	Department of Virology, Faculty of Biology, Lomonosov Moscow State University Address: Leninskie Gory 1-12, Moscow, Russian Federation	**Novel approach for the design of universal recombinant vaccines to control diseases caused by viruses with high antigenic variability**
P32	Erica	Sanna	Department of Life and Environmental Sciences, University of Cagliari; A Building, Cittadella Universitaria di Monserrato, S.P. 8 km 0.700, 09042, Cagliari	**Probing Potential Pan-hCoV Inhibitors by Targeting Nsp13 Helicase**
P33	Claudia	Storti	Department of Public Health, Experimental and Forensic Medicine, Università di Pavia	**Mucroporin-M1 analogues: synthesis, conformation and structure-activity correlation**
P34	Daniele	Volpin	University of Padova, Via Francesco Marzolo n°1 (35131, Padova)	**Targeting West Nile virus replicases: NS3 and heterodimers inhibitors**

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Plenary lectures abstracts

2024.09.23

Broader-acting antivirals for epidemic and pandemic preparedness

Johan Neyts
Laboratory of Virology and Antiviral Research, Rega Institute, KU Leuven, Belgium

Broad(er)-acting antiviral drugs, active against entire genera or families of viruses, should be developed and stockpiled in epidemic/pandemic peacetime. They can be used to counter outbreaks as soon as the new virus has been identified and will also remain important pharmacological tools after the introduction of vaccines and monoclonal antibodies. I will discuss strategies to develop such antiviral drugs and give example for a selected number of genera/viruses.

NOTES

2024.09.23

A Biochemist's Journey of Antiviral Drug Discovery

Joy Feng
Ph.D, San Francisco, CA

In the antiviral drug discovery field, targeting key viral enzymes involved in the replication machinery has yielded great success; however, a compound's safety profile is often the determining factor in its success. In Joy's talk, she will share three stories on drug discovery from a biochemist point of view: the mechanism of action on drug combination synergy, the development of a comprehensive screening paradigm for early detection of drug toxicity, and discovery of remdesivir, the first FDA approved antiviral to treat COVID-19. Joy will also share the lessons she learned as she looking through the different stages of her career.

NOTES

2024.09.24

Using DNA-Encoded Library Screening to Effectively and Rapidly Develop New Antiviral Drugs

Damian W. Young

Center for Drug Discovery, Department of Pathology and Immunology and Department of Biochemistry and Molecular Pharmacology, Baylor College of Medicine, One Baylor Plaza, Houston TX, USA, 77030

The traditional process of drug discovery generally begins with the High-Throughput Screening (HTS) of around 1-million small molecules to identify starting points to a disease target. However, HTS is a long and costly process that has a high failure rate. These aspects make HTS particularly challenging for developing antiviral agents against emerging viral diseases. Rather than using HTS as an entry point to new antiviral drugs, we have investigated the use of DNA-Encoded Libraries (DELs), a technology based on screening billions of drug-like compounds that are each covalently attached to a unique DNA sequence. DEL screening is achieved by subjecting an epitope-tagged viral target to the multibillion membered DEL as a single pooled mixture. The target protein serves as bait to capture binders from the complex mixture by removing the protein through the epitope tag. The structural identity of the binders to the viral target is revealed through Next Generation Sequencing of their DNA tags. The rapid and low cost of DEL screening, coupled with the greater efficacy associated with accessing a larger chemical space of billions of compounds makes the DEL paradigm well suited to antiviral drug discovery. The lecture will focus on the application of DELs to SARS-CoV-2 drug discovery.

NOTES

2024.09.24

Challenges and opportunities in the management of antiviral drug resistance among DNA viruses

Graciela Andrei

Rega Institute for Medical Research, KU Leuven, Belgium.

Opportunistic viral infections represent a well-recognized complication in immunocompromised patients. In particular, infections caused by herpesviruses are associated with significant morbidity and mortality in this group of patients. A hallmark of herpesviruses is their ability to establish latent infections and to reactivate when the immune system of the host is compromised or fails. When the host immune system is weakened, herpesviruses (either following primary infection or reactivation) can cause persistent life-threatening infections that require prolonged antiviral therapy, facilitating the emergence of drug resistance. Recent advances in rapid diagnostic methods and the introduction of potent antiviral compounds have made it possible to establish efficient management strategies for several herpesviruses. Despite their medical importance, only a few antivirals are approved for prophylaxis and/or treatment of herpesvirus diseases and traditional anti-herpesvirus drugs target the viral DNA polymerase. Most of these compounds have important toxicities and drug-resistance is a critical issue. More recently, drugs targeting the cytomegalovirus terminase, the UL97 protein kinase or the herpes simplex helicase-primase have been approved or are undergoing clinical trials. Although the newer anti-herpesvirus drugs appears to be well tolerated, they show low genetic barrier to resistance. Alternative antiviral regimens are preferred to treat herpesvirus infections in order to avoid cumulative toxicity and selection of multidrug resistant virus. A phenotypic and/or genotypic characterization of herpesvirus accompanied by a rational use of antiviral agents and other chemotherapeutics is essential to reduce toxic and expensive treatments. In order to adapt rationally antiviral therapy, surveillance of herpesvirus drug-resistance is highly recommended in immunocompromised patients and in immunocompetent individuals who suffer from infections in immune privileged sites [such as the central nervous system (CNS) and the eye)]. Infections of the CNS with herpesviruses are life threatening and require rapid therapeutic decisions in all type of patients. Development of new anti-herpetic compounds acting on a different viral target than the DNA polymerase that have a good safety profile remains a crucial need. The development of new drugs is challenging, time-consuming, expensive, and is not a priority for pharmaceutical companies due to a poor cost-benefit. Infections caused by other DNA viruses other than herpesviruses, such as adenovirus and polyomavirus, are associated with severe disease in transplant recipients. Successes and failures in development of anti-herpesvirus drugs will be discussed as well as new alternative targets to inhibit herpesviruses and poxviruses, another family of large DNA viruses, which has been recently in the spotlight because of the global outbreak of Mpox (formerly monkeypox) in 2022. Zoonotic poxviruses are also another threat to human health, providing a supplementary argument to search for new anti-poxvirus drugs.

2024.09.24

First in Class and Best in Class in antiviral drug discovery processes, two different meaning, challenges and opportunities

Vincenzo Summa

Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II

First in class and Best in class are the definitions associate to very important drugs. The lecture will present the discovery of two historical Direct Acting Antiviral Agents (DAAA) that have been associated with these nomenclatures. Raltegravir1 and Grazoprevir2 acting as HIV integrase inhibitor, the first, and HCV protease inhibitor, the second. The two drugs were developed in two different scenarios, each one was approached with different strategies and solutions to the problems raised during the development. The presentation will describe the key points of the drug discovery processes of the two drugs, highlighting the creative medicinal chemistry approaches that led to the approval of the two innovative Direct Acting Antiviral Agents.

1) Discovery of Raltegravir, a Potent, Selective Orally Bioavailable HIV-Integrase Inhibitor for the Treatment of HIV-AIDS Infection. V. Summa, A. Petrocchi, Fa. Bonelli, B. Crescenzi, M. Donghi, M. Ferrara, F. Fiore, C. Gardelli, O. Gonzalez Paz, D. J. Hazuda, P. Jones, O. Kinzel, R. Laufer, E. Monteagudo, E. Muraglia, E. Nizi, F. Orvieto, P. Pace, G. Pescatore, R. Scarpelli, K. Stillmock, M. V. Witmer, M. Rowley. J. Med. Chem. 2008, 51, 5843–5855
2) MK-5172, a Selective Inhibitor of Hepatitis C Virus NS3/4a Protease with Broad Activity across Genotypes and Resistant Variants, V. Summa, S. W. Ludmerer, J. A. McCauley, C. Fandozzi, C. Burlein, G. Claudio, et all Antimicrob Agents Chemother 2012 Aug;56(8):4161-7

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2024.09.25

Outflanking the Pathogenic Arenaviruses

Brian B. Gowen

Department of Animal, Dairy, and Veterinary Sciences and Institute for Antiviral Research, Utah State University, Logan, Utah, United States

Arenaviruses are enveloped, bi-segmented single-strand RNA viruses that cause zoonotic infections in humans with various outcomes ranging from mild febrile illness to life-threatening hemorrhagic fever. Despite significant advances in the understanding of how arenaviruses cause disease, measures to prevent or combat infection and disease are limited. A focus of the Gowen lab is the development of preventative and therapeutic interventions against pathogenic arenaviruses. This talk will explore the vulnerabilities of arenaviruses and review recent advances toward a multi-faceted approach for treating arenaviral disease.

NOTES

2024.09.25

HIV: to integrate, or not to integrate, that is the question

José G. Dekker, Bep Klaver, Atze T. Das and Ben Berkhout

Laboratory of Experimental Virology, Amsterdam UMC, the Netherlands

During my career in HIV research, we witnessed several unusual virus evolution events. For instance, we reported that HIV can become resistant to the nucleoside drug 3TC by a mutation in the catalytic core of the RT enzyme. This M184V substitution also had a small impact on viral fitness by reducing the processivity of the RT polymerase. We described a drug-resistance mechanism for the entry inhibitor T20, but also an exotic route towards drug-dependence. When we moved to antiviral strategies using RNA-interference (RNAi), we reported simple resistance by a point mutation in the targeted viral genome sequence. Mutations outside the actual target sequence were selected on rare occasions, which turned out to cause resistance by inducing a new and stable RNA fold that resists RNAi attack. More recently, we probed CRISPR-Cas as antiviral strategy, which is meant to inactivate the HIV genome by introduction of mutations (edits) in critical HIV DNA genome sequences. This worked out beautifully, but again the virus had an answer by selecting resistant HIV genomes with CRISPR-induced mutations that do not interfere with virus replication. The new integrase inhibitor Dolutegravir (DTG) seems to avoid all these problems and drug-resistance was also not reported in clinical studies. As we doubted that HIV could not acquire DTG-resistance, we performed massive in vitro virus evolution experiments. We observed that HIV can acquire resistance to DTG by skipping the HIV DNA integration step, which is considered one of the hallmarks of retrovirus replication. This perplexing result confirms an early finding by the Delelis laboratory. We will present the underlying mechanism and discuss the clinical relevance.

How polypurine tract changes in the HIV-1 RNA genome can cause resistance against the Integrase inhibitor Dolutegravir. Atze T. Das and Ben Berkhout, mBio 2018

Mutations in the HIV-1 3′-polypurine tract can confer Dolutegravir resistance.
José G. Dekker, Bep Klaver, Ben Berkhout and Atze T. Das Antimicrob Agents Chemother 2022

HIV-1 3′-polypurine tract mutations confer Dolutegravir resistance by switching to an integration-independent replication mechanism via 1-LTR circles. José G. Dekker, Bep Klaver, Ben Berkhout and Atze T. Das, J Virol 2023

NOTES

2024.09.25

Exploiting Ebola virus suppression of the innate immune activation as target for drug development

Angela Corona

Laboratorio di Virologia molecolare, Dipartimento di Scienze della Vita e dell'Ambiente. Università degli Studi di Cagliari. Cittadella Universitaria di Monserrato. SS-554. Monserrato. Italy

The Ebola virus (EBOV) is a highly infectious and lethal pathogen responsible for recurrent clusters of Ebola virus disease (EVD), whose outbreaks have become increasingly frequent and large. Even with several promising therapeutic candidates under investigation, there is a lack of agents to treat the infection effectively. Therefore, efforts are devoted to elucidating all the possible targets and mechanisms to find new therapeutic opportunities. The EVD onset is linked to the ability of EBOV to efficiently suppress the innate immune system at the early stages of infection.
The main factors in this suppression are viral protein 35 (VP35) and viral protein 24 (VP24). VP35 is a multifunctional protein which plays a major role in the EBOV life cycle as a potent immune antagonist by inhibiting the dsRNA-induced activation of the RIG-I signaling pathway, suppressing IFN-beta production, while VP24 exerts its action by binding the NPI-1 subfamily of karyopherin-α proteins, involved in the nuclear transport of phosphorylated STAT1 protein, thus blocking the transcription of IFN-stimulated genes.
In this lecture I will present how molecular virology and cell-based mechanistic studies revealed how both VP35 and VP24 proteins can be efficiently targeted, identifying ligands able to restore the innate immune response, and how the restoration or pharmacological amplification of the innate immune response has been shown to overcome the EBOV replication.

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2024.09.25

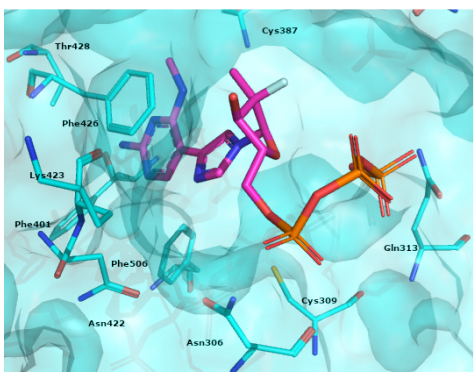
Flex-Nucleosides – A Strategic Approach To Broad-Spectrum Antiviral Therapeutics

Prof. Kathie Seley-Radtke

Department of Chemistry & Biochemistry University of Maryland, Baltimore County (UMBC), Baltimore, MD

The severity and rapid spread of the coronavirus pandemic served to drive home that we were completely unprepared to fight such an outbreak. As a result, it became clear that there was a critical need for small molecule, orally bioavailable, broad-spectrum drugs that could be stockpiled and readily distributed when the next outbreak occurs. In that regard, for many years nucleos(t)ides have maintained a prominent role as one of the cornerstones of antiviral and anticancer therapeutics, and numerous scaffolds in nucleos(t)ide and nucleic acid drug design have been pursued. One such approach involves adding flexibility to the sugar moieties of nucleos(t)ides, for example, in the highly successful anti-HIV/HBV drug Tenofovir developed by Antonín Holý. In contrast, introduction of flexibility to the nucleobase scaffold has only more recently gained significance with the invention of our fleximers. This modification has led to a significant improvements in antiviral activity, and in some cases endowing the nucleoside with potent broad-spectrum activity across several viral families, when the parent rigid nucleoside was inactive. Another advantage observed is the ability to avoid resistance mechanisms related to point mutations by engaging secondary amino acid residues not previously involved in the mechanism of action. A brief history of their development, and recent antiviral findings for this innovative class of nucleos(t)ides will be discussed.

Illustration of Flex-AT-527-TP in the CoV-2 Nsp-14/Nsp10 complex.



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2024.09.26

Development of immunotherapy based on genetically delivered Human endogenous retrovirus-K based VLPs

Peter Johannes Holst,

Chief Science Officer, HERVOLUTION Therapeutics & Associate Professor at the department of Biomedical Research at the University of Copenhagen.

During controlled chronic viral infections antigen B cells are required to sustain CD4+ T cells that support ongoing B cell and CD8+ T cell immunity. Human endogenous retroviruses expressed within cancers has a unique potential to reconstitute effective immunity by combined cancer expression, exosome incorporation and exosome display of viral envelope proteins, and may provide a quantitatively important contribution to cancers spontaneously forming tertiary lymphoid structures (TLS). The cancer intrinsic antigenic properties can also be exploited for immunization by genetic delivery of virus-like-particle forming Gag antigens co-expressed with surface expressible envelope proteins. Such antigen targeting endogenous murine leukemia viruses antigens of mice, and expressed in viral vectors induce a modest T cell response and a weak and irregular B cell response. However, point mutations in the putative ISD domain within the viral envelope protein further stimulate T cell responses and allow effective responses against growing tumors. While encouraging, lack of potent B cell responses conflicts with reports of spontaneous immunity towards human endogenous retrovirus K in lung cancers and our internal data demonstrating effective break-of-tolerance against HERV-K in transgenic mice and against simian ERV-K in non-human primates, as well as therapeutic induction of TLS-like structures. We observe that HERV-K forms VLPs with highly dense and regular display of envelope spikes and HERV-K like VLPs can be used to generate chimeric VLPs capable of inducing augmented B cell responses against disparate human ERVs and rapidly break B cell tolerance towards murine leukemia viruses. Collectively, ISD mutations and chimeric VLPs thus allow effective break of B and T cell tolerance for HERV targeted immunotherapy.

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2024.09.26

Data informed drug discovery, software tools and case studies

Phill Gribbon

Fraunhofer-Institut für Translationale Medizin und Pharmakologie ITMP
Department Discovery Research Hamburg, Germany

In pre-clinical research, the use of advanced data analysis methods contribute to the overall goal of defining the physico-chemical properties, effectiveness, PK/PD, ADME and toxicity properties of therapeutic agents. For a chemical biologist the use of methods such as knowledge graphs and machine learning allows for predictions of binding, potency, selectivity and mechanism of action. Thus, for the efficient application for the use of ML in drugs and chemical probes discovery, there is a need for data, metadata and analysis workflows from the fields of computational chemistry, bioinformatics, chemical biology, drug discovery, medicinal chemistry, preclinical research and human disease biology to be accessible and interoperable. This lecture will focus on use-cases based in the field of compound repurposing to illustrate the operation of data-driven drug discovery.

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2024.09.26

Isolation and Characterization of Neutralizing Monoclonal Antibodies from a Large Panel of Murine Antibodies against RBD of the SARS-CoV-2 Spike Protein

Emanuela D'Acunto

Takis Biotech

The COVID-19 pandemic, once a global crisis, is now largely under control, a testament to the extraordinary global efforts involving vaccination and public health measures. However, the relentless evolution of SARS-CoV-2, leading to the emergence of new variants, continues to underscore the importance of remaining vigilant and adaptable. Monoclonal antibodies (mAbs) have stood out as a powerful and immediate therapeutic response to COVID-19. Despite the success of mAbs, the evolution of SARS-CoV-2 continues to pose challenges, and the available antibodies are no longer effective. New variants require the ongoing development of effective antibodies. Here, we describe the generation and characterization of neutralizing mAbs against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein by combining plasmid DNA and recombinant protein vaccination. By integrating genetic immunization for rapid antibody production and the potent immune stimulation enabled by protein vaccination, we produced a rich pool of antibodies, each with unique binding and neutralizing specificities, tested with the ELISA, BLI and FACS assays and the pseudovirus assay, respectively. Here, we present a panel of mAbs effective against the SARS-CoV-2 variants up to Omicron BA.1 and BA.5, with the flexibility to target emerging variants. Furthermore, some of these antibodies are also capable of cross-binding SARS-CoV-1. This findings opens up the intriguing possibility of using our panel of monoclonal antibodies, as a powerful tool against an eventual coronavirus related pandemic event using by massive and rapid screening to identify a neutralizing antibody of the circulating variant ready to be translated in a therapeutic tool for viral neutralization. This approach ensures the preparedness principle is in place to address SARS-CoV-2 actual and future infections.

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2024.09.27

Innovative strategies for antiviral development: from viral RNA to attachment mimicry

Valeria Cagno

Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Switzerland

Despite significant efforts in drug development, the number of available antivirals remains limited. This issue is particularly acute for respiratory viruses, which cause millions of infections annually. For these viruses, antivirals often have a narrow window of effectiveness and a high risk of resistance development. To address this challenge, my lab focuses on identifying molecules capable of mimicking attachment receptors, binding to, and destroying viruses in the extracellular environment. This approach has led to the discovery of potent inhibitors effective against Influenza virus, RSV, SARS-CoV-2, and Parainfluenza virus in cells, human-derived tissues, and mice. Additionally, we are developing inhibitors targeting structured elements in viral RNA. Notably, we have identified inhibitors of the programmed ribosomal frameshifting element in SARS-CoV-2 that exhibit a high barrier to resistance. Our ongoing efforts are focused on developing more potent derivatives and targeting structured elements within the Picornaviridae family.

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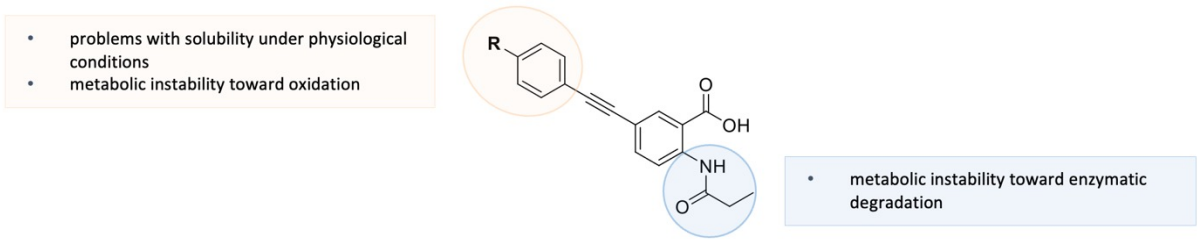
2024.09.27

Anthranilic Acid based Dihydroorotate Dehydrogenase Inhibitors for the Use as Antiviral Agents

Chris Meier

University of Hamburg, Germany

Dihydroorotate dehydrogenase (DHODH) is an enzyme involved in the de novo pyrimidine synthesis leading to the formation of uridine monophosphate (UMP). UMP is then converted to the other pyrimidine nucleotides needed for the biosynthesis of RNA and DNA. In resting cells, the demand for pyrimidines is sufficiently covered by the salvage pathway, whereas fast proliferating cells, like cancer cells or also virus replicating cells, additionally depend on the de novo pyrimidine synthesis. The need for a high supply of pyrimidine nucleotides is required for a fast viral replication renders the enzyme DHODH as a promising target for the development of new antiviral agents. Inhibition of DHODH leads to reduced levels of pyrimidine nucleotides that primarily affect the replication of the viral genome. DHODH inhibitors function as host factor targeted antiviral agents that provide several advantages compared to inhibitors targeting single viral enzymes, such as acting as potential broad-spectrum antivirals which provide a high barrier to resistance.[1,2] We have developed different series of DHODH inhibitors based on anthranilic acids that showed broad-spectrum antiviral activities against several RNA viruses, including bunyaviruses, flaviviruses and filoviruses.[3] Herein, we describe structural optimizations using an identified lead structure of the anthranilic acid series, especially with regard to the enzymatic stability and aqueous solubility. The objective of this study is to improve the drug-like properties of the selected compounds without the loss of antiviral efficacy.



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2024.09.27

Cellular APOBEC3 enzymes and herpesviruses – why we should care!

Reuben Harris

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Howard Hughes Medical Institute, University of Texas Health San Antonio, San Antonio, TX 78229, USA.

Viruses have evolved diverse mechanisms to counteract host innate immune effector proteins such as the APOBEC3 family of DNA cytosine deaminases. Lentiviruses use a poly-ubiquitination/degradation mechanism. In contrast, we have found that gamma-herpesviruses such as Epstein-Barr virus (EBV) and Kaposi's Sarcoma Herpesvirus (KSHV) utilize the large subunit of the viral ribonucleotide reductase (vRNR) to bind to nuclear APOBEC3B, inhibit access to viral single-stranded DNA substrates, and shuttle the ternary complex into cytoplasmic aggregates, far away from nuclear sites of lytic-phase viral DNA replication. The mechanism of DNA deamination inhibition is explained by cryo-EM structures demonstrating sequestration of the substrate binding loops of APOBEC3B by the vRNR. We have additionally found that this direct interaction is conserved in gamma-herpesviruses that infect humans and Old World monkeys but is completely absent in viruses that infect New World monkeys. Strategies to prevent the vRNR from neutralizing APOBEC3B have the potential to enable natural innate immune restriction of herpesvirus infections and help treat diseases such as infectious mononucleosis (EBV), cold sores and genital herpes (HSV-1/2), shingles (VSV), and longer-term consequences of herpesvirus infection such as multiple sclerosis (EBV) and cancer (EBV, KSHV).

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2024.09.27

Tools and Tactics for Targeting RNA with Small Molecules”

John S. Schneekloth, Jr. (Jay)

Chemical Biology Laboratory National Cancer Institute, NIH
Frederick MD, USA

Although some 85% of the human genome is transcribed into RNA, just 3% of those sequences code for proteins. Extensive efforts in genomics have revealed that noncoding RNAs regulate diverse biological outcomes, including for a variety of disease states. In addition to the human transcriptome, viral genomes have been found to encode structure and function in unique ways, representing attractive targets for small molecules. In this seminar I will discuss my group’s longstanding efforts to identify small molecules that bind to and alter the function of viral and bacterial genomic elements, and how these efforts provide evidence for future studies developing novel antibacterial, antiviral, and anticancer medicines.

NOTES

Selected talks abstracts

O.1 Design and Synthesis of Novel Macrocycle Inhibitors Targeting SARS-CoV-2 Main Protease

*Alessia Alberico (1), Enzo Tramontano (2), Angela Corona (2), Carmen Cerchia (1), Raffaele De Francesco (3), Luca Guidotti (4), Vincenzo Summa (1).

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Viral proteases are primary targets to develop efficacious and safe antiviral therapy thanks to their essential role in viral replication. With the COVID-19 pandemic outbreak, caused by SARS-CoV-2, drug discovery efforts focused on the development of Main Protease (Mpro) inhibitors as Nirmatrelvir,[1] the first in class covalent reversible peptidomimetic inhibitors of SARS-CoV-2 Mpro. Mpro exhibits high sequence identity in the substrate binding site across the Orthocoronavirinae subfamily,[2] allowing the identification of Pan CoVs inhibitors. To identify a novel class of Mpro inhibitors, our rational design started from of macrocyclic peptidomimetics reported as human rhinovirus (HRV) 3C protease inhibitors.[3] Both HRV and SARS-CoV-2 proteases recognize Gln in the substrate P1 residue, therefore, similarly to HRV inhibitors, we insert Proline at P1 replacing the common γ-lactam ring in Mpro inhibitors. The Proline retained the key H-bond with the His163 in the S1 binding site of Mpro, similarly to the His161 of HRV 3C protease. We synthesized, through SPPS, three sets of novel macrocycles, varying P2 residues, P3 capping groups, and macrocycles ring sizes, endowed with a nitrile warhead, acting as covalent reversible inhibitors. These macrocycles displayed micromolar IC50 in biochemical assays against SARS-CoV-2 and MERS Mpro, laying the foundation for additional structural optimization aimed at developing a novel class of broad-spectrum Coronaviruses Mpro inhibitors.
References: 1) Owen D.R. et al. 2021. Science 374,1586-1593. 2) Cannalire R. et al. 2022. J. Med. 65, 4, 2716–2746. 3) Namoto, K et al. 2018. Bioorganic & Medicinal Chemistry Letters, 28(5), 906-909.

O.2 Studies on the SARS-CoV-2 Mpro dimerization mechanism using a split luciferase assay

*Renee Delgado(1)

1) The University of Health Science Center 7703 Floyd Curl Drive San Antonio, TX 78229

The main protease enzyme Mpro of SARS-CoV-2 cleaves the viral polyprotein into functional units responsible for virus replication and pathogenesis. Interestingly, Mpro functions only as a homodimer. However, the structure-function relationship and conformational plasticity of the Mpro dimerization mechanism remains poorly understood. To address these gaps in knowledge, I have developed quantitative luciferase-based reporter assays for Mpro dimerization in living cells and in vitro. We propose to study the Mpro dimerization interface through the construction and analysis of a panel structure-guided and evolution-informed mutants, as well as through targeted deep-mutational scanning. Additionally, these novel assays also enable studies on the mechanism of inhibitor-facilitated dimerization. Altogether, the proposed studies will yield new assays for studying coronavirus protease biology, unique insights into the mechanisms of Mpro dimerization and its allosteric modulation, and novel compounds that can be used as chemical probes of these molecular mechanisms.

0.3 Discovery of novel non-nucleoside inhibitors against SARS-COV-2 minimal replication-transcription complex

*Paolo Malune (1), Daniela Iaconis (2), Candida Manelfi (2), Stefano Giunta (1), Valentina Piras (1), Andrea Beccari(2), Enzo Tramontano(1), Francesca Esposito(1).

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After its emergence in late 2019, SARS-CoV-2 rapidly caused a global threat, with over 7 million deaths from the infection all over the world, as reported by WHO as of June 2024. The ssRNA(+) viral genome of around 30 kbs encodes for 16 functional proteins, 4 structural and several accessory ones, and it is one of the longest among RNA viruses. The replication of the genome and transcription of subgenomic mRNAs is mediated by nsp12, which acts in complex with nsp7 and nsp8 forming the replication-transcription complex. In order to discover new safe and effective drugs against this virus, in collaboration with Dompé farmaceutici, two large libraries of safe-in-man and natural compounds were screened in silico against the SARS-CoV-2 nsp12, nsp7 and nsp8 complex by targeting the orthosteric and two allosteric sites of nsp12. Almost 300.000 screening hits were selected by docking score, novelty on the enzymatic target and repurposed compounds were also filtered by clinical phase. The best 119 hit compounds were evaluated for their ability to inhibit SARS-CoV-2 RdRp activity in a PAGE-based biochemical assay. Several compounds showed promising inhibitory activity, with IC50 values in the low micromolar range. Since some of these compounds appeared to dock on allosteric sites only, competition assays are on-going to confirm and validate allosteric sites on SARS-CoV-2 nsp12. Cell-based assays to evaluate the efficacy of the hits on SARS-CoV-2 replication are in progress.

0.4 The in vitro genetic barrier to resistance of lenacapavir is not affected by viral subtype or heavy treatment exposure

Chiara Paletti (1*), Niccolò Bartolini (1), Federica Giammarino (1-2), Francesco Saladini (1), Ilaria Vicenti (1), Lia Fiaschi (1), Camilla Biba (1), Ilenia Varasi (1), Federico Garcia (3), Anne-Genevieve Marcelin (4), Maurizio Zazzi (1), Vincenzo Spagnuolo (5), Emanuele Focà (6), Stefano Rusconi (7).

1) Department of Medical Biotechnologies, University of Siena, Siena, Italy. 2) Division of Infectious Diseases, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden. 3) Hospital Universitario Clinico San Cecilio, Clinical Microbiology, Granada, Spain; Instituto de Investigación Ibs. Granada, Spain; Ciber de Enfermedades Infecciosas, Ciberinfec, Madrid, Spain. 4) Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpital Pitié-Salpêtrière, Laboratoire de virologie, Paris, France. 5) Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy. 6) Unit of Infectious and Tropical Diseases, Department of Clinical and Experimental Sciences, University of Brescia and ASST Spedali Civili di Brescia, Brescia, Italy. 7) Infectious Diseases Unit, ASST Ovest Milanese, Legnano General Hospital and DIBIC, University of Milan, Milan.

Introduction. Lenacapavir is the first-in-class HIV-1 capsid inhibitor approved for clinical usage. This study evaluated the susceptibility and the genetic barrier to resistance to lenacapavir in B and non-B subtypes from therapy-naïve (TN) and HTE PWH. Methods. Phenotypic susceptibility to lenacapavir was measured using TZM-bl cells with recombinant viruses harboring GAG-PR from TN and HTE plasma samples. Fold-change (FC) IC50 values were calculated with respect to the wild-type NL4-3 strain. In vitro resistance selection (IVRS) experiments were performed by exposing MT-2 cells infected with recombinant viruses to increasing concentrations of lenacapavir until viral breakthrough was observed at 100X IC50 or after 105 days from the start of the IVRS. Sanger sequencing of the p24 coding region was performed at each viral breakthrough. Results. Lenacapavir baseline susceptibility was comparable between HTE vs. TN (median FC 0.9 [IQR 0.3-1.6] vs. 1.6 [0.6-3.0], p=0.253, Mann-Whitney test) and between B vs. non-B subtypes (median FC 0.7 [0.2-2.2] vs. 1.6 [0.7-2.5], p=0.141). Time to viral breakthrough was comparable among both B vs. non-B subtypes and HTE vs. TN PWH at 10X (p=0.112 and p=0.551, respectively, log-rank test) and 100X IC₅₀ (p=0.226 and p=0.382, respectively). Known lenacapavir resistance mutations emerged in 25/27 cultures and the non-polymorphic aminoacid substitutions F169L, V86M and E213D were detected in three distinct cases. Conclusions. The genetic genetic barrier to resistance to lenacapavir was not affected by viral subtype, previous failures to other ARV classes or long-time exposure to ART. Frequent detection of emerging mutations indicates a low genetic barrier to resistance.

O.5 New strategies for inhibiting Hepatitis B Virus entry using Cyclosporine A analogues

M. Longo Minnolo (*1,2), L. Donnici (2), L. G. Guidotti (3,4), R. Di Fabio (4) and R. De Francesco (1,2)

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Chronic hepatitis B virus (HBV) infection is still a significant global health issue. Although antiviral drugs can suppress HBV replication, eradication remains challenging due to the persistence of the viral genome in infected cell nuclei. A novel therapeutic approach involves entry inhibitors, such as Cyclosporine A (an immunosuppressant with a cyclophilin A-dependent mechanism of action) targeting HBV receptor, NTCP. This study investigates new Cyclosporine A analogues as potential entry inhibitors against HBV and its satellite virus HDV, both utilizing NTCP. In vitro assays were used to evaluate antiviral activity of the compounds against HBV, HDV, and HCV. Our CsA analogues effectively inhibit HBV and HDV entry and a competition receptor assay with a fluorescently labeled viral peptide confirmed that they prevent NTCP-mediated viral attachment. We indirectly demonstrated that they act via a cyclophilin A-independent mechanism showing no activity against HBV or HCV replication. Additionally, a luciferase reporter system was used to assess the immunosuppressive effect, showing a reduced immunosuppressive effect compared to Cylosporine A. Finally, the analogues impact on NTCP bile acid transport function was evaluated using an NBD-labeled bile acid, showing limited selectivity and denoting the need for further improvements. In conclusion, we identified a novel series of Cyclosporine A analogues with potent antiviral activity, up to 100-fold more potent than Cyclosporine A. These compounds inhibit virus attachment and entry by interacting with NTCP. Future work will focus on enhancing selectivity and conducting in vivo studies to analyse pharmacokinetics and pharmacodynamics.

O.6 Exploring thiazole systems for antiviral applications: synthesis and biological evaluation

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A small library of [1,3]thiazolo[5,4-h][1,6]naphthyridines has been synthetized through a multistep procedure, with the aim of assessing their antiviral activity as potential anti-CoVs agents. Biochemical assays on SARS-CoV-2 Nsp13 helicase revealed interesting derivatives with inhibitory activity at micromolar level. Based on these results, in the attempt of obtaining more potent compounds, a scaffold hopping strategy has been applied leading to a series of decorated N-(2-phenyl-1,3-thiazol-5-yl)benzamides. The newly synthesized compounds were also evaluated in cell-based assays with a panel of different viruses. Interesting results were obtained in both naphthyridine and N-(2-phenyl-1,3-thiazol-5-yl)benzamide series. Indeed, one [1,3]thiazolo[5,4-h][1,6]naphthyridine derivative emerged as a lead candidate for further investigations due to its strong antiviral activity against human coronavirus HCoV-229E, showing an EC50 value of 0.85 μM. Within the N-(2-phenyl-1,3-thiazol-5-yl)benzamide series, two compounds proved to potently inhibit the replication of influenza virus A/H1N1 with EC50 values in the submicromolar range for different A/H1N1 strains. Pseudovirus entry assay in MDCK cells confirmed their ability to inhibit the H1N1 HA-mediated entry process. Results will be discussed.
References: [1] M.W. Hull, et al., Annals of Medicine 2011, 43(5), 375-388. [2] F.S. Hosseini, et al., Life Sciences 2020, 258, 118205. [3] A. Birkmann, et al., Journal of Medicinal Chemistry 2022, 65(20), 13614-13628.

O.7 Investigating the role of lake microbial enzymes on virus inactivation

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Enteroviruses are a group of single-stranded RNA virus that are associated with multiple diseases in humans and animals. Their transmission relies on their ability to persist in the environment. In aquatic environments, multiple abiotic and biotic factors contribute to viral inactivation. While abiotic factors such as temperature, pH and UV-light exposure have been studied, biotic factors remain uncharacterized. Our lab has previously shown that lake water microbial communities reduce the infectivity of Echovirus-11 (E11) and Coxsackievirus-A9 (CVA9), and that this reduction is associated with the production of bacterial proteases. Here, we aim to further characterize these bacterial communities and their role in viral inactivation. We have developed a pipeline to isolate and grow bacterial communities from Lake Geneva using chemostats. Chemostats are open culture systems with a continuous inflow of nutrients and removal of microbial metabolic waste, allowing bacterial cultures to be maintained in exponential growth phase for an extended period of time. Once the bacterial communities are established, we will characterize their viral inactivation capacity, and their community structure and metabolism. To characterize bacterial capacity to inactivate enteroviruses, E11 and CVA9 will be inoculated into the chemostat communities and viral infectivity will be monitored over time. In addition, to characterize our lake communities and their metabolism, a combination of "-omics" and biochemical approaches will be used. Metagenomics will be used to infer the taxonomic profiles of the communities, metatranscriptomics their gene expression profiles, and metabolomics their metabolite production. Data analysis will be focused on identifying bacterial taxa and metabolic processes responsible for viral inactivation in the chemostat communities. All in all, our research provides a foundation to better understand the fate of enteroviruses in the environment and their interaction with environmental microbial communities.

O.8 Targeting HSV-1 through FASN inhibitors: implications for Alzheimer's Disease

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Herpes simplex virus type-1 (HSV-1) relies on host metabolic pathways for replication, posing challenges for antiviral strategies. Due to its neuroinvasive and neurotoxic nature, HSV-1 has been linked to neurodegenerative diseases, including Alzheimer's disease (AD). This study investigates the relationship between HSV-1 infection and cellular metabolism to develop effective antiviral interventions and identify potential AD prevention targets. Using SH-SY5Y neuronal-like cells infected with HSV-1, we established an in vitro model of HSV-1-associated neuronal pathologies. Cells were treated with fatty acid synthase (FASN) inhibitors (CMS121, C75) to evaluate their antiviral effects. Moreover, FASN gene expression was silenced using specific short hairpin RNA to rule out off-target effects. The impact of FASN inhibitors on Aß-like plaque formation was assessed using a 3D tissue culture model simulating herpesvirus-induced AD. HSV-1 infection altered lipid levels and increased FASN expression, affecting viral infectivity and host-cell interactions. CMS121 and C75 significantly reduced HSV-1 infectivity and inhibited Aß-like plaque formation in the 3D AD model, suggesting a link between HSV-1-mediated lipid dysregulation and AD pathology. These results highlight the potential of FASN inhibitors as promising antiviral agents targeting lipid metabolism in HSV-1 infection and AD. Our findings demonstrate the efficacy of FASN inhibitors, CMS121 and C75, in reducing HSV-1 infectivity and inhibiting Aß-like plaque formation. This suggests a novel antiviral approach, highlighting lipid metabolism as a therapeutic target for both HSV-1 infection and AD. Future research should focus on elucidating the mechanisms and exploring the clinical potential of these compounds.

O.9 Novel Fusion Inhibitors Targeting Hemagglutinin (HA) of Influenza A H1N1 Virus

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Each year, influenza A virus leads to millions of severe cases globally. The scarce clinically available antivirals combined with the global transmission of virus resistant to these drugs, point the urgent need of novel anti-influenza compounds directed to new targets and/or distinct mode of action with respect to the currently available drugs. We previously discovered a unique class of N-benzyl-4,4-disubstituted-piperidines that specifically inhibit H1N1 influenza A virus fusion[1]. Mechanistic and computational studies with the peptidomimetic prototype DICAM180 revealed that its inhibitory activity is mediated by binding to a previously unexplored pocket in the HA2 subunit, close to the highly conserved fusion peptide. The proposed binding mode of these compounds involves a direct π-stacking interaction with the Phe9 residue of the HA2 fusion peptide. DICAM180 interacts with only one of the fusion peptides of the monomers in the homotrimeric HA. This binding mode has served as started point for designing new derivatives capable of establishing an additional interaction with the fusion peptide of the second monomer of HA. These modifications aim to achieve simultaneous interaction with the Phe9 residues of both monomers, enhancing antiviral activity and developing broad-spectrum anti-influenza compounds. Several chemical modifications of DICAM180 have been designed using a combination of docking studies and Molecular Dynamics simulations to create compounds able of these additional interactions and to improve activity data.
References:

[1] De Castro, S. et al. N-benzyl 4,4-disubstituted piperidines as a potent class of influenza H1N1 virus inhibitors showing a novel mechanism of hemagglutinin fusion peptide interaction. Eur. J. Med. Chem.2020,194, 112223.

O.10 Fusion inhibitory peptides inhibit SARS-CoV-2 Omicron BA.5 transmission in hamsters

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Since its emergence in 2019, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) continues to circulate and cause coronavirus disease-2019 (COVID-19). Vaccines have been effective in protecting from severe disease. However, SARS-CoV-2 continues to evolve antigenically, necessitating vaccine updates. Potent therapeutics that are impervious to antigenic drift and can inhibit SARS-CoV-2 transmission could therefore play a crucial role in dampening the COVID-19 outbreak. We have previously shown that fusion inhibitory peptides, which interfere with the conformational rearrangement of the spike protein, can prevent transmission of the 2020 variant of SARS-CoV-2 in ferrets. Here, we assessed the activity of optimized lipopeptides with longer half-lives and improved solubility against Omicron BA.5 transmission in hamsters. First, we determined the minimized dose in a low-volume inoculum required for reproducible infection and viral shedding of male and female hamsters. Next, we determined the optimal co-housing duration for direct-contact transmission. Finally, we compared the potency of optimized lipopeptides QE-PEG24-chol and QE7-PEG24-chol with that of [SARSHRC-peg4]2-chol in preventing transmission. Inoculation of hamsters with 103 TCID50Omicron BA.5 led to reproducible infection. Shedding of

infectious virus was significantly higher in males. Compared to the replication kinetics of ancestral SARS-CoV-2, Omicron BA.5 inoculation led to lower viral loads and less weight loss. To ensure 100% direct-contact transmission from inoculated donor hamsters to naïve recipients, co-housing between 24 and 48 hours post inoculation of the donors was optimal. After evaluation of multiple lipopeptides in vitro, the most promising candidates QE-PEG24-chol and QE7-PEG24-chol were evaluated in the transmission model. [SARSHRC-peg4]2-chol prevented transmission in 6 out of 6 recipients when given at high dose (25 mg/kg). At a 10-fold lower dose QE-PEG24-chol and QE7-PEG24-chol both prevented transmission in 5 out of 6 recipients. Taken together, the potency of optimized lipopeptides encourages further investigation. Fusion inhibitory peptides remain promising candidates to be used as a prophylactic intervention against variants of SARS-CoV-2.

O.11 Elucidation of the pan-flavivirus potential of NS4B-targeting antivirals

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Several flaviviruses, such as dengue (DENV) or yellow fever virus (YFV), pose a significant medical and socioeconomic burden due to their widespread prevalence and severe health impact. Other members of this virus family may receive less attention today, but the unexpected Zika virus outbreak in 2015-2016 demonstrated that they too might pose the risk of epidemics with serious public health consequences. Despite the obvious need, there are currently no antiviral drugs available to treat flavivirus infections, and we are inadequately prepared for future epidemics or pandemics. The most advanced drug candidates are the DENV-specific compounds NITD-688 and JNJ-1802, which are in Phase 2 clinical trials. Using a photoaffinity labeling compound with high structural similarity to JNJ-1802, we demonstrated binding to DENV non-structural protein 4B (NS4B) and its precursor NS4A-2K-NS4B, consistent with the clustering of resistance mutations in the corresponding coding region of the viral genome. We also found that the DENV NS4B inhibitor blocked the de novo formation of vesicle packets (VPs), the sites of viral RNA replication, which is functionally linked to a blocked interaction between the NS2B/NS3 protease/helicase complex and the NS4A-2K-NS4B cleavage intermediate. Using a combination of molecular dynamics simulations and further experiments, we are currently investigating whether other flaviviruses also possess NS4A-2K-NS4B functionality in VPs formation and, if so, whether this could be exploited for the development of pan-flavivirus NS4B inhibitors.

O.12 Exploiting the potency of new DKA derivatives as SARS-CoV-2 nsp13 inhibitors and their role in blocking viral replication

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The outbreak of the novel coronavirus SARS-CoV-2 in late 2019 has presented an unprecedented global health challenge and highlighted the risk of a zoonotic spillover into human population, bringing the attention on the development of coronaviruses antivirals. For RNA viruses, RNA helicases have been recognized to play critical role in viral replication cycles and showed a high sequence identity among all known coronaviruses. Considering that SARS-CoV-2 helicase (nsp13) lacks homologous proteins in humans and other mammals, it could be a good target for discovering selective antiviral inhibitors. Nsp13 is a multidomain enzyme able to unwind DNA or RNA in an NTP-dependent manner with a 5'-3' polarity. It couples two C-terminal RecA ATPase domains, characteristic of the 1B (SF1B) helicase superfamily, with other three domains: the N-terminal zinc-binding domain (ZBD), essential for the helicase activity, a stalk, and a 1B domain. In the present study, we exploit the new class of indolyldiketoacids (DKA) derivatives recently identified as nsp13 inhibitors, starting from the optimization of our previous DKA scaffolds exhibiting broad-spectrum antiviral activity. The class of new compounds was tested on both the SARS-CoV-2 nsp13 unwinding and ATPase associated activities. Many of them showed the capacity to inhibit both nsp13 enzymatic functions in a significant low micromolar range and were selected for the evaluation of their activity in blocking SARS-CoV-2 replication. Moreover, DKAs provided a strong rationale to evaluate their potential broad-spectrum antiviral activity, testing their capacity to block viral replication of other alpha- and beta-coronaviruses.

O.13 Polyoxometalate exerts broad-spectrum activity against human respiratory viruses hampering viral entry.

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Human respiratory viruses have an enormous impact on national health systems, societies, and economy due to the rapid airborne transmission and epidemic spread of such pathogens, while effective specific antiviral drugs to counteract infections are still lacking. Here, we identified two Keggin-type polyoxometalates (POMs), [TiW11CoO40] 8 , TiW11Co) and [Ti2 PW 10 O 40] 7,(Ti2PW10), endowed with broad-spectrum activity against enveloped and non-enveloped human respiratory viruses, i.e., coronavirus (HCoV-OC43), rhinovirus (HRV-A1), respiratory syncytial virus (RSV-A2), and adenovirus (AdV-5). Ti 2PW10,†showed highly favorable selectivity indexes against all tested viruses (SIs >700), and its antiviral potential was further investigated against human coronaviruses and rhinoviruses. This POM was found to inhibit replication of multiple HCoV and HRV strains, in different cell systems. Ti2PW10,did not affect virus binding or intracellular viral replication, but selectively inhibited the viral entry. Serial passaging of virus in presence of the POM revealed a high barrier to development of Ti2PW10 resistant variants of HRV-A1 or HCoV-OC43. Moreover, Ti2PW10, was able to inhibit HRV-A1 production in a 3D model of the human nasal epithelium and, importantly, the antiviral treatment did not determine cytotoxicity or tissue damage. A mucoadhesive thermosensitive in situ hydrogel formulation for nasal delivery was also developed for Ti2PW10. Overall, good biocompatibility on cell lines and human nasal epithelia, broad-spectrum activity, and absence of antiviral resistance development reveal the potential of Ti2PW10, as an antiviral candidate for the development of a treatment of acute respiratory viral diseases, warranting further studies to identify the specific target/s of the polyanion and assess its clinical potential.

O.14 A novel class of SARS-COV-2 nsp13 Helicase and NTPase inhibitors: Pyridobenzothiazolone (PBTZ)-based compounds

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Since 2020, the scientific community made significant strides against SARS-CoV-2, granting Emergency Use List to ten vaccines and approving three direct-antiviral agents: Nirmatrelvir as main protease inhibitor, Molnupiravir and Remdesivir as polymerase inhibitors. The present work is focused on less explored SARS-CoV-2 nsp13 helicase, thanks to its sequence identity across Coronaviruses could be considered for Pan-CoVs antivirals development [2] A Pyridobenzothiazolones (PBTZs) library, known as low to sub-μM pan-flavivirus polymerase inhibitors, was investigated as CoVs inhibitors.The library was tested against SARS-CoV-2 in VeroE6 at 30mM, the non-toxic and most active compounds were titered and tested against the key enzymes, main protease, polymerase, helicase, in enzymatic assays. All the active compounds specifically inhibit nsp13 unwinding and NTPase functionalities. HeE1-2r emerged as a promising hit: IC_{50} (Helicase assay) ~ 0.32 μM, IC50 (NTPase assay) ~ 4.4 μM, EC50 (VeroE6-GFP) ~ 10.37 μM. Further investigation led to the design of novel analogs exploring different moieties of the PBTZs common scaffold. Since there was no co-crystal structure available, the design relied on a ligand-based structure-activity relationship study. Two derivatives, F2F-2020406 and F2F-2020400, showed improved activity compared to the original hit. Our results identified PBTZs as a new class of low-μM nsp13 inhibitors with antiviral activity in cell-based assay, opening a full exploration of the series as Pan-Helicase CoVs inhibitors.

References:WHO EUL/PQ status of COVID-19 Vaccines, WHO, 2024; Corona et al. ACS Pharmacol. Sci. 2022, 5, 226–239
Cannalire et al., Antiviral Research, 2019, 6-1

O.15 Amphibian peptide HS-1 and its ala-Scanning analogues as broad-spectrum antivirals

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The escalating threat of viral infections requires the exploration of novel therapeutic agents. Current antiviral strategies can target specific viruses or, alternatively, broad-spectrum antiviral agents able of combating diverse viral pathogens are investigated. Antimicrobial peptides (AMPs) have emerged as promising candidates, due to their multifaceted antimicrobial properties. Exploring the rich arsenal of bioactive compounds derived from amphibian skin secretions, this study aims to investigate the antiviral activity of HS-1, a peptide isolated from the skin secretion of Hypsiobas semilineatus. Additionally, an ala-scanning mutagenesis of HS-1 has been performed to identify which are the key residues and to find out the lead peptide endowed with the lower cytotoxicity and the increased antiviral effect. Peptides were synthesized utilizing the solid-phase Fmoc chemistry approach, followed by purification through reversed-phase HPLC. Ala-scanning mutagenesis was employed to generate HS-1 analogues, systematically replacing each residue with alanine. The evaluation of cytotoxicity was conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Antiviral activity was evaluated across a spectrum of viruses, including enveloped, naked, DNA, and RNA viruses, by plaque reduction assays and molecular test. Preincubation of HS-1 peptide with viruses has a significant antiviral activity, indicative of its possible ability to disrupt viral envelopes. The peptide interferes with the extracellular phases of viral lifecycle, potentially impeding viral attachment and entry. Ala-scanning mutagenesis has unveiled the key residues essential for antiviral activity. Notably, all HS-1 analogues have a reduced toxicity profile, with some peptides endowed with an enhanced antiviral efficacy compared to the native peptide HS-1. These findings highlight the promising perspective of amphibian skin peptides as antiviral agents. Future investigations will delve into elucidating the mechanism of action of HS-1 derived analogues, thereby elucidating their therapeutic potential in combating viral infections.

O.16 Multi-Omics Profiling to Unveil West Nile Virus interactions with Infected cells

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West Nile Virus (WNV) is a mosquito-borne flavivirus that causes a wide range of symptoms, from mild febrile illness to severe neuroinvasive diseases. Understanding the molecular mechanisms and host-pathogen interactions underlying WNV infections is crucial for developing effective therapeutics and preventive measures. Using in-vitro experiments with a susceptible cell line, we applied a multi-omics approach integrating transcriptomics, proteomics, metabolomics, and lipidomics to gain a comprehensive view of the complex biological processes involved in WNV pathogenesis. Analyses revealed differential expression of 968 genes, most of which are involved in the immune response, confirmed by the modulated translation of 78 proteins, while 10 proteins were found without corresponding upregulated transcripts. Metabolomic and lipidomic profiling indicated significant alterations in amino acid and energy metabolism pathways, particularly related to pyruvate, and notably decreased levels of triacylglycerol and modulation of phosphatidylethanolamine classes, suggesting a metabolic shift and membrane rearrangement in infected cells. Another intriguing aspect involves human endogenous retroviruses (HERVs), remnants of ancient viral infections within the human genome. HERVs can be reactivated by viral infections, potentially influencing disease outcomes. During WNV infection, differential expression of 42 specific HERV loci was observed, potentially modulating immune responses and contributing to pathogenesis, partly due to their co-localization with important human genes, such as IFIT1, 2, and 5. This multi-omics approach not only advances our understanding of WNV pathogenesis but also sheds light on the enigmatic role of HERV activity in viral infections, laying the foundation for innovative strategies to combat emerging infectious diseases.

Lee HJ et al. Early cellular and molecular signatures correlate with the severity of West Nile virus infection. iScience. 2023 Nov 2;26(12):108387. doi: 10.1016/j.isci.2023.108387.

O.17 Discovery of thioaryl imides as a family of SARS-COV-2 ENTRY inhibitors

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The COVID pandemic caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) ended in May 2023 according to the WHO yet the virus still remains a global threat [1]. This was possible thanks to RNA vaccines that target fragments of the spike-shaped protein (S-Protein) and block the entry of the virus into the host cells; unfortunately, this efficacy could be compromised by the appearance of new mutations. Due to this the development of new molecules effective against COVID by targeting the entry of the virus is a matter of high interest in medicinal chemistry [2].In this context, we report herein the preliminary evaluation of a library of synthetic thioaryl imides through a high-throughput screening (HTS) assay based on VSV-S pseudoparticles harboring the XBB.1.5 S protein of SARS-CoV-2 in Vero E6 cells expressing the TMPRSS2 entry factor according to the method described by Gargantilla et al.[3]. The assayed compounds proved a noticeable ability to inhibit the virus attachment to the host cells in the model system, showing IC50 values in the range 1-7 µM whereas keeping a safety profile (CC50>50 µM). These promising results drove us to initiate a Structure-Activity Relationship study directed to provide a bigger library of structurally-related derivatives with enhanced activity and selectivity.

References:[1] World Health Organization (WHO) report, COVID-19 epidemiological update, Edition 167, 17 May 2024.[2] European Centre for Disease Prevention and Control, SARS-CoV-2 variants of concern as of 31 May 2024, (https://www.ecdc.europa.eu/en/covid-19/variants-concern).[3] Gargantilla, M. et al. J. Med. Chem., 2023, 66, 10432

O.18 Molecular evolution of the hepatitis B virus in the presence of some promising antiviral plant extract formulations

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Chronic hepatitis B virus (HBV) infection currently remains one of the major global health burdens, affecting around 292 million persons worldwide. The current treatments interferons (IFNs) and nucleos(t)ide analogues (NAs) do not eliminate the virus due to the persistence of covalently closed circular DNA (cccDNA) in infected cells. Owing to the adverse effects and resistance developed with current treatments, medicinal plants have been investigated to cure hepatitis B. Caffeoylquinic acids and their derivatives are reported poses antiviral activity against various viruses and hepatoprotective properties. Many aspects of the underlying mechanisms are still enigmatic. This project aims to evaluate the effect of Isochlorogenic Acid A (ICAA) on HBV viral and subviral particles and HBV-infected hepatocytes in detail. Cell culture-based systems (HepAD38, HepG2, and Huh7.5) were used to investigate HBV viral and subviral particles by qPCR, ELISA, western blot, density gradient fractionation, and confocal microscopy upon ICAA treatment. Additionally, Next-generation sequencing was used to assess transcriptomic change in HepAD38. We observe a significant decrease of secreted HBeAg, HBsAg, HBV DNA, and intracellular HBV transcripts. Assembly of HBV virion was also affected by ICAA. Transcriptome analysis using KEEG pathway revealed that there is a significant impact of ICAA in HBV replicating cells on pathways modulating tumorigenesis. These data will help deepen our understanding of the mechanism of action of ICAA on HBV and HBV-related pathologies

O.19 Prevention of lethal SARS-CoV-2 replication in human ACE2-transgenic mice through error prone suggests that daclatasvir dose can be adjusted for early COVID-19 therapy

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The 2019 pandemic associated with the coronavirus (COVID-19), caused by SARS-COV-2 (severe acute respiratory syndrome coronavirus 2) has been causing more than 100,000 deaths per month globally and leaving long-term morbidities in millions of people. Due to the continuous worldwide circulation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants with improved transmissibility and ability to escape the humoral immune response elicited by vaccines or prior infection, antiviral drugs are still necessary to treat patients with 2019 coronavirus disease (COVID-19). The main class of antiviral drugs used in the last decades, nucleoside/nucleotide analogs, have demonstrated limited clinical benefit against COVID-19. Although this class of compounds inhibits the SARS-CoV-2 RNA polymerase when incorporated into the newly synthesized virus genome, they may be excised by this virus's proofreading exonuclease. Thus, in this study we investigate an repositioned exonuclease inhibitor that can effectively inhibit, in vitro and in vivo, the replication of SARS-COV-2 and the immunology effects of this experimental treatment in transgenic mices. We examined the hepatitis C virus (HCV) nonstructural protein 5A (NS5A) inhibitor daclatasvir for potential repurposing against SARS-CoV-2. Based on molecular docking, enzymatic assays and cellular-based studies, evidence was provided in favor of these compounds possessing anti-exonuclease activity. To further test the hypothesis that SARS-CoV-2 exonuclease is susceptible to HCV NS5A inhibitors, we evaluated if inhibition of exonuclease by these NS5A inhibitors would result in more mutations in the virus genome. Virus genetic diversity was catalogued under the selective pressure of these drugs. The NS5A inhibitor daclatasvir all increased the number of mutations in the SARS-CoV-2 genome, in special A to U and C to U changes were enhanced by almost 60 times. Daclatasvir was also tested in vivo because of its higher plasma exposure in humans compared to the other compounds. By also inducing error-prone in vivo replication, daclatasvir prevented lethal SARS-CoV-2 replication in transgenic mice expressing human ACE2 (K18-hACE-2 mouse strain), reduced cell death and inflammatory responses at a concentration that could allow further clinical development against COVID-19.

O.20 Evaluation of broad-spectrum piperazine-based compounds able to inhibit flavivirus and/or SARS-CoV-2 replication in a live virus assay.

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Background: No specific antiviral therapy for Zika virus (ZIKV), Dengue virus (DENV) is available and only two drugs available for SARS-CoV-2 (Nirmatrelvir, NRM and Remdesivir). This study aimed to evaluate newly synthesized compounds (CMPs) for their antiviral potential against these viruses.
Materials and Methods: CMPs, designed with a piperazine ring as central core, underwent in vitro testing using live virus cell-based assays. Two compound families were developed: one with 2-phenyl piperazine (1°family, 1-29) and the other with unsubstituted piperazine (2°family, 30-51). Once assessed the 50% cytotoxic drug concentration (CC_{50}), the non-toxic doses were administered to lung, A549 ACE-2 TMPRSS-2 (A549-AT) and hepatoma Huh7 cells and challenged with viral stocks at 0.001 MOI. Inhibitory activity was measured and expressed as half-maximal inhibitory concentration (IC_{50}) and selectivity index (SI) was calculated.
Results: The median CC50 of the 1°family was 61.5 µM (Huh7) and 138 µM (A549-AT), and the 2° family showing values of 400.0 µM (Huh7) and 129.9 µM (A549-AT). CMP 50 demonstrated broad activity against ZIKV and SARS-CoV-2, while nine CMPs showed activity against ZIKV, with four also active against DENV (35, 39, 41 and 42). Compounds with IC50;15 µM underwent further analysis.
Conclusions: CMP 50 showed promising broad-spectrum activity against SARS-CoV-2 and ZIKV, although less effective against SARS-CoV-2 compared to NRM. CMPs 41, 42, and 49 effectively inhibited ZIKV, with CMP 42 holding potential against both ZIKV and DENV. Piperazine-based compounds represent a potential class of pan Flavivirus agents, addressing the urgent need for effective treatments for ZIKV and DENV infections.

O.21 AMALPHI: A Machine Learning Platform for Predicting Drug-Induced PhospholIpidosis

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Phospholipidosis (PLD) is a condition marked by abnormal phospholipid buildup within cells, notably in lysosomes1, impacting diverse tissues. This disorder is commonly triggered by cationic amphiphilic drugs (CADs). Recent research has underscored significant links between CAD lipophilicity, their capacity to induce PLD, and their antiviral efficacy against various viruses such as hepatitis C virus (HCV), Japanese encephalitis virus (JEV), Epstein–Barr virus (EBV), and severe acute respiratory syndrome coronavirus (SARS-CoV)2. During the COVID-19 pandemic, a publication in Science3 emphasized PLD's pivotal role in drugs exhibiting anti-SARS-CoV-2 activity. It was noted that many compounds showing in vitro antiviral activity induce PLD, potentially leading to misleading positive results. In response, we developed a series of Machine Learning classifiers to preemptively identify the potential of drug candidates to induce PLD. These classifiers were based on 545 highly curated small molecules extracted from ChEMBL v30. The top-performing model, using the Balanced Random Forest algorithm, demonstrated remarkable performance, achieving an AUC value in validation of 0.90. These findings prompted us to make this model freely accessible through a user-friendly web platform named AMALPHI (https://www.ba.ic.cnr.it/softwareic/amalphiportal/), serving as a valuable resource in antiviral research to conduct early assessments of the PLD inducer potential 4.
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O.22 Plant-derived Antiviral Strategies: Antiherpetic Properties of Tomato Root Exudate and Antimicrobial Efficacy of Soybean-Derived plasticizer

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The growing prevalence of viral infections and the rise of drug resistance to current treatments urgently call to identify new antiviral strategies. Plant-based products are envisaged as alternative sources of molecules helpful for developing innovative antiviral molecules and materials. Phytochemicals have traditionally been used to treat various diseases, and research into biologically active compounds from plants is increasing, as they offer the potential for more sustainable treatments and safer drugs with fewer side effects. Within this frame, our work shows the antiherpetic properties of the organic-soluble fraction of a root exudate derived from the tomato plant (Solanum lycopersicum) and the antimicrobial properties of a new plasticizer, named GDE, obtained from the esterification and epoxidation of soybean fatty acids. Our results demonstrate that the root exudate from Solanum lycopersicum displays notable efficacy against two prominent members of the family Herpesviridae, specifically herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV), by interfering with a molecular event during the viral replication phase, while GDE effectively damage enveloped viruses, such as HSV-1 and the β-coronavirus prototype HCoV-OC43, due to the interference and disruption of the viral lipid layer. Overall, these data unveil two novel natural products endowed with antiviral activity, presenting promising and valuable alternatives to existing treatments.

O.23 Structural and Functional Characterization of Receptor Binding Domain (RBD) for the reconstructed HERV Envelopes and their interaction with Host Cellular Receptors

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Endogenous Retroviruses (ERVs) are the remnants of the ancient viral infections that are accumulated in the genomes of almost all the vertebrates. Over the years it has been observed that most of these ERV elements degenerates' overtime and become non-functional, but the human genomes still contain complete ERV provirus that still restore the full infection capabilities. A general proviral structure is composed of three genic portions: (i) the group specific gag gene, (ii) the polymerase (pol) genes encoding viral enzymes and (iii) the envelope (env) gene encoding the envelope glycoprotein. This internal region is flanked by the long terminal repeats (LTRs) at both the ends. Among the different retroviral proteins, Env ones are the most relevant to human biology that interacts with the specific cell surface receptors of the host and mediates the viral fusion and entry into host cell. Most of the HERV sequences have eventually become inactive due to either the disruption of the open reading frames (ORFs) by mutations or completely losing the viral genes by recombination through various factors and hence we reconstructed 32 Env sequences representing the prototypes of these ancestral proteins in Class I, Class II, and Class III HERVs. The SU subunit of the Env glycoprotein consists of a receptor-binding domain (RBD), which is responsible for the recognition of the host cellular receptor(s) and is considered more variable. Hence, the reconstructed HERV Envs were used to identify the receptor binding domain and build a 3D structure to further analyze its fusogenicactivity. In the present study, we determined the structure of RBD of the reconstructed HERV Env proteins that shows structural similarities with the leukemia viruses despite of having less sequence similarities. Lastly, to identify the cellular surface receptors for reconstructed HERV Env we implemented literature survey search through which we obtained a list of potential receptors. Overall, by combining various approaches we aim to better understand the fusogenic activity of the HERVs with the host cellular receptors.

O.24 Anchimerically Activatable Prodrugs of Remdesivir Nucleotide Monophosphate with Enhanced Metabolic Stability for Oral Delivery

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Remdesivir (RDV) is an adenosine analog nucleotide prodrug with broad-spectrum activity against RNA viruses. RDV received full FDA approval to treat COVID-19 in 2020 but requires intravenous administration which severely limits its utility. RDV relies on carboxyesterase enzymes for activation to RDV monophosphate (RDV-MP). The high expression levels of these enzymes in the liver contribute to rapid first-pass metabolism and the resulting poor oral bioavailability. Obeldesivir (OBV) is a prodrug of the RDV parent nucleoside (GS-441524) with substantially improved oral bioavailability and is currently in Phase III clinical trials for the treatment of COVID-19. Nonetheless, developing a RDV-MP prodrug with good oral bioavailability remains an attractive goal as RDV consistently demonstrates higher in vitro potency than OBV and GS-441524. This improved potency is attributed to the rate-limiting first phosphorylation step required to produce the ultimate active RDV-triphosphate metabolite. We are reporting two phosphoramidate prodrugs of RDV-MP that replace the ester moiety of RDV with a thioether group which chemically self-activates via anchimeric assistance. These compounds display comparable potency to RDV against the SARS-CoV-2 Omicron subvariant BA.5 in vitro. Our compounds show good aqueous solubility and high mouse plasma stability. Additionally, we have identified CYP3A4 as the primary metabolizer of our compounds in mouse liver S9 fraction. Further, we have demonstrated that co-administration of ritonavir, a selective CYP3A4 inhibitor currently used in the FDA-approved COVID-19 therapeutic PAXLOVID TM, fully rescues the in vitro liver stability of the reported compounds. We anticipate further development of these RDV-MP prodrugs for in vivo pharmacokinetic analysis.

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Poster Abstracts

P.1 Viral Communities in Antarctic lake core samples

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My current research on the Antarctic virome, still in its early stages, focuses on exploring the diverse and unique viral communities in the extreme environments of Antarctica. I am investigating the viral diversity in ice core samples from Antarctic lakes. Utilizing advanced metagenomic techniques, I aim to understand how these viruses interact with their hosts, contribute to nutrient cycles, and influence microbial community dynamics. Although in its initial phase, this research has the potential to provide insights into viral evolution, explore biotechnological applications, and examine the role of viruses in climate change. My goal is to enhance our understanding of global viromes and the resilience of life in extreme conditions. This research also carries potential medical applications, including the insights into virus-host interactions that could inform the development of new therapeutic strategies.

P2. RHDV–S domain based subviral particle formation

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Rabbit Hemorrhagic Disease Virus (RHDV) is a lethal pathogen of domestic and wild rabbits (Oryctolagus cuniculus) that causes enormous economic losses and irreparable damage both directly and indirectly to Mediterranean ecosystems. RHDV is a non-enveloped virus containing single-stranded positive-sense RNA genome. The viral capsid comprises 180 copies of the major structural protein (VP1). RHDV, like many caliciviruses, cannot be propagated in cell cultures, and much of our structural understanding of these viruses is based on studies conducted with virus-like particles (VLPs) that result from the self-assembly of the VP1 protein following expression in heterologous systems, notably in insect cells using baculovirus vectors. Structurally, the VP1 is divided into the N-terminal (S) domain and the protruding C-terminal domain (P), which are joined by a short and flexible hinge. It has been shown that the heterologous expression of S domain of human norovirus using baculovirus self-assemble into nanoparticles equivalent to the inner smooth layer of the capsid (Bertolotti-Ciarlet et al., 2004). In this study, recombinant RHDV S domain, maintaining the hinge, and with the addition of a histidine tail for an easier detection and purification, was tested for its ability to form subviral nanoparticles. In addition to the wild type sequence, three mutants (N71C, S126C, 4CYS) were obtained by substitution of different amino acids with cysteines to determine if the formation of disulphide bonds could confer additional stability to the nanoparticles. The ability of these proteins to form subviral structures was analysed and optimized purification protocols were determined.

P.3 Unveiling new scaffolds for the inhibition of West Nile NS3 helicase

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Flaviviruses are vector-borne RNA viruses that cause serious diseases in human populations such as encephalitis for West Nile or haemorrhagic fever for Dengue virus. To date, no antiviral treatments are available. To overcome the high mutation rate and the rapid selection of resistance toward common antiviral drugs, we focused our drug discovery efforts on non-structural protein 3 (NS3), helicase domain, because it is well-conserved (67% sequence identity) across all Flaviviruses. NS3 contains a serine-protease domain at its N terminus and an ATP-driven helicase and RNA triphosphatase at its C-terminal end. NS3 is essential for viral RNA synthesis; in fact, viruses carrying a defective or impaired NS3hel gene cannot replicate properly. NS3hel requires RNA-stimulated NTPase activity to provide the energy for RNA unwinding and translocation along the dsRNA. By means of Computer-Aided Drug Design (CADD) approaches we have identified a series of compounds characterised by the dihydropyrazole scaffold capable of inhibiting the activity of the NS3hel at low mM concentration.

P.4 Exploring the broad-spectrum antiviral potential of Bithiazole derivatives targeting PI4KB

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The development of effective therapies for emerging and re-emerging viruses is hindered by several challenges, including the lack of antiviral drugs with broad-spectrum activity, time-consuming development timeline for new vaccines and the emergence of viral resistance to common antiviral therapies targeting viral proteins. An alternative strategy is to target host factors that are essential for viral replication of multiple viruses but dispensable for host cell viability. PI4KB is a host lipid kinase that plays an essential role in the replication of a number of positive-strand RNA viruses, particularly those belonging to the Picornaviridae family, making it a potential target. We evaluated a new library of bithiazole derivatives as PI4KB inhibitors that proved to be active in the nanomolar range and exhibited a broad-spectrum activity against viruses of different families, including Coronaviridae, Flaviviridae and Poxviridae. Mutation in 2B protein of RVA16, known to confer resistance to PI4KB inhibitors, reduced the activity of the compounds, confirming PI4KB targeting. Nonetheless, the most active compounds retained activity in the submicromolar range against RVA16 and inhibited additional viruses (e.g. ZIKV, MPOXV) that are not affected by common PI4KB inhibitors (e.g. Enviroxime). This behavior suggests a multitarget mechanism of action for the bithiazole antivirals. Finally, the best compounds showed synergy with rupintrivir and, most importantly, impeded the selection of rupintrivir resistance mutations. Overall, our compounds demonstrated strong viral inhibition and absence of toxicity, supporting the development of bithiazole derivatives as broad-spectrum antivirals.

P.5 Development of a virus-free cell assay for the evaluation of viral protease inhibitors

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Development of a new antiviral drugs targeting a specific enzyme starts with in vitro screening of compounds activity on the recombinant target. Subsequently, the most promising molecules are evaluated for their ability to impair viral replication in a cell-based infection assay, which imposes a high level of biosafety that is precluded to most laboratories. To attenuate this bottleneck, we decided to develop a virus-free cellular assay to further screen inhibitors of viral proteases, limiting the number of active compounds to be tested in cell-based virus replication assay.
We initially focused on Sars-CoV-2 Main protease (Mpro), which is essential for viral replication. We fused the two NanoBiT luciferase subunits (Promega) into a single construct separating them with a flexible spacer containing an Mpro cutting sequence. The expression of Mpro causes cleavage of the luciferase, resulting in loss of light emission. Consequently, Mpro inhibitors restore the light signal proportionally to their potency, as we demonstrated using the reference compound GC376. We observed an IC50 value of 9.43 ± 1.69 μM, which is well within the limits of values reported in the literature.

P.6 Identification of specific HERV loci differentially expressed in Multiple Sclerosis patients as potential biomarkers and therapeutic targets

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Human Endogenous Retroviruses (HERVs) represent about 8% of our genome, originating from ancient infections. Several studies tentatively linked HERV expression to multiple sclerosis (MS) and an antibody against HERV-W Envelope protein (Env) is under clinical trial for MS therapy. Despite the evidence suggesting that HERV-W is involved in MS, the specific HERV-W loci differentially expressed in MS patients remain unknown. This study hence aims to identify HERV loci differentially expressed in MS patients to validate them as potential biomarkers and therapeutic targets. For this, we generated and compared RNA sequencing data from peripheral blood mononuclear cells (PBMCs) and monocytes of 80 MS patients and 40 healthy controls, with a dedicated pipeline for HERV-derived transcriptome. Total RNA was extracted and sequenced from both, followed by clustering analysis, validation of differentially expressed HERVs (deHERVs) and assessment of their diagnostic potential for MS. HERV expression was shown to be modulated by MS and influenced by gender. In females with MS, 122 loci were differentially expressed, compared to 19 in males. 12 deHERV were in common, including 4 upregulated loci. A focus on upregulated deHERVs revealed that 18 retain intact open reading frames (ORFs) that could potentially produce retroviral proteins, including 11 Env. Three HERV-W elements in chr 2q13, 6q23.3, and 12q24.33 are specifically upregulated in females and our study led to patenting of specific locus, 2q13. The study overall provides an exhaustive description of HERV loci modulation in PBMCs and monocytes in MS, identifying specific HERV loci with coding potential to be tested for their possible role in the disease.

P.7 Harnessing the innate immune response through NOD1 agonists prevents SARS-CoV-2 infection in human lung epitelial cells

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The difficulty to exert a direct antiviral activity in the lung, is one of the major roadblocks for the management of respiratory infections, such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Boosting the innate immune response of the respiratory mucosa at early stages of the infection is a potential alternative intervention for the treatment of respiratory infections. Amongst the immunomodulators tested, NLR agonists TriDAP (NOD1) and M-TriDAP (dual NOD1/2) showed the best potency and selectivity, inducing up to 3.3-fold increase of IL8+ cells in a dose-dependent manner, without impairing cell viability. Response to NOD1 and dual NOD1/2 agonists was 2-fold-higher compared to LPS control. NOD1 and dual NOD1/2 agonist activity involved NF-κB and ISRE pathways induction. NOD1downregulation (siRNA) resulted in a 93% reduction of IL-8+ cells cocultured with TriDAP or M-TriDAP. Selective NOD1 and NOD1/2 inhibitors impaired the NOD1-induced activation of NF-κB and ISRE pathways. PBMCs were unresponsive to NOD1 agonists, suggesting tissue specific activity of NOD1 in lung epithelial cells, without a global systemic activation. Finally, NOD1 agonist Tri-DAP and dual NOD1/2 agonist M-TriDAP, promoted an antiviral environment that prevented SARS-CoV-2 replication in lung epithelial cells (57% protection). This work provides the biological basis for the development of host-directed therapies based on the NLR pathway to boost the innate immune system for an early viral clearance and infection resolution.

P.8 Characterisation of the mechanisms of interferon production inhibition by Ebola virus VP35 wild-type and mutants

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The deadly disease resulting from Ebola virus (EBOV) infection frames this filovirus as a threat to global health. Among the proteins encoded by the genome, the viral protein 35 (VP35) is one of the most interesting targets. VP35 is a multifunctional protein involved in the replicative cycle of the virus as a polymerase cofactor, but also known for targeting the interferon β (IFNβ) production as a mechanism of immune evasion. It has been reported that VP35 binds dsRNA shielding it from the cellular sensor RIG-I while interacts at the same time with many cellular factors involved in the interferon production cascade, including TBK1 and IRF3. We wanted to investigate the relevance of the single interactions starting from a structure-based alanine scanning of the full-length VP35 to determine which are the residues majorly involved in these interactions. In particular, we investigated some VP35 end-capping residues, such as F239, Q274, I278, Q279, K319, R322 and K339, that have been found to play a key role in the dsRNA binding. We obtained recombinant proteins and performed biochemical assays to analyze the ability of the mutants to bind dsRNA from which resulted a loss of dsRNA binding capacity by many of them. Consequently, we performed cell-based assays to evaluate the effect of the same residues on the IFNβ production inhibition, observing that many mutants retained their IFNβ antagonist abilities. We next aim to dissect the VP35 mutants' interactions with the single components of the IFNβ production cascade by super-resolution imaging analysis.

P.9 The expression of Human Endogenous Retroviruses in PBMC is modulated by SARS-CoV-2 acute infection and shows a specific transcriptional pattern as compared to other COVID-19 clinical stages

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The modulation of Human Endogenous Retroviruses (HERV) is able to sustain innate immune activation in various infectious and inflammatory contexts due to the expression of immunogenic viral transcripts and, eventually, immunogenic proteins. SARS-CoV-2 infection is known to stimulate an important inflammatory response, which characterizes COVID-19 pathogenesis. To gain a better understanding on this poorly defined interplay, we performed the high-throughput sequencing and differential expression analysis of ~3300 HERV loci in the peripheral blood mononuclear cells (PBMC) of 37 individuals with acute SARS-CoV-2 infection and 8 healthy controls (HC). PBMC have been chosen as these cells are not directly infected by the virus but have a crucial role in the inflammatory and immune events defining the COVID-19 pathogenesis. Results showed that SARS-CoV-2 infection modulates HERV expression and allows to clearly divide infected individuals from HC in unsupervised clustering analyses. Differential expression analyses confirmed that a total of 359 HERV loci were significantly modulated in the presence of SARS-CoV-2 infection and 253 of them were upregulated. The obtained transcriptional signature during SARS-CoV-2 acute infection has been also compared with previous results obtained in the PBMC from convalescent and retesting positive patients, revealing a specific pattern of HERV modulation but also a subset of HERV loci significantly modulated in all COVID-19 clinical stages. The present study shows a comprehensive picture of the HERV transcriptome in PBMC and its modulation in HC as compared to different COVID-19 clinical stages, thought to be relevant to the disease clinical manifestation and outcome.

P.10 Diarylheterocycle: in silico design throught Molecoular Modelling studies of a new potentially inhibitors active against main protease of Sars-CoV-2.

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The Covid-19 pandemic has prompted the scientific community to develop new weapons against viral infections. Starting from an existing class of compounds and enhancing it can be an excellent starting point for designing new potential antivirals. Literature studies have shown that Mofezolac and SC-560, known as COX-1 inhibitors, can weakly interact with the main protease of SARS-CoV-2. Starting from in silico studies of ligand-protein interactions with this protease could be a promising strategy. This study aims to search databases for compounds similar to the templates and screen them using molecular modeling software. The FLAP software provides a comprehensive view of the interaction areas involved, the amino acid residues involved, and the 3D and 2D poses of each ligand. Virtual screenings indicate that 8 molecules exhibit higher interaction scores than SC-560 and 7 molecules show higher values than Mofezolac. To validate these in silico studies, the pocket where the protease inhibitor N3 is co-crystallized was chosen, and Mofezolac and SC-560 were included in the created database to compare interaction scores.

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P.11 Recombinant nucleoside-2´-deoxyribosyltransferase Lactobacillus delbrueckii application for green nucleoside analogs production

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Nowadays, in the process of the production of biologically active substances, including drugs, the replacement of chemical with chemical-enzymatic or exclusively enzymatic syntheses using genetic engineering strains that produce target enzymes is clearly visible. Nucleoside-2´-deoxyribosyltransferase (NDT) (E.C. 2.4.2.6) catalyzes reversible transglycosylation between a purine and/or pyrimidine 2′-deoxynucleoside (donor) and a purine and/or pyrimidine base (acceptor). NDT plays a key role in the production of nucleosides analogues due to its high chemo-, regio- and stereoselectivity. Recombinant NDT was used for synthesis of promising antiviral drugs (didanosine, vidarabine, trifluridine, zalcitabine) as well as antitumor drugs (fludarabine, nelarabine, clofarabine, floxuridine, cladribine). 6-thio-2′-deoxyguanosine (6TDG) is a promising antitumor agent with a broad spectrum of action. We assume that in addition to antitumor activity, 6TDG will serve as antiviral agent since thio-nucleosides inhibit viral replication. The work aim was to create a strain-producer of NDT and use this enzyme in the one-step synthesis reaction of 6TDG. A strain of the bacterium Lactobacillus delbrueckii was chosen as the source of the gene encoding the protein sequence. The genetic construction pET42a-ndt was obtained using genetic engineering manipulations. On this basis, the recombinant E. coli strain pET42a-NDT, producing nucleoside-2´-deoxyribosyltransferase, was successfully created. We demonstrated the possibility of using this enzyme for the enzymatic synthesis of the 6TDG with yield of 93–95 %.

P.12 Exploring the structural features for the identification of ZIKV NS3pro allosteric inhibitors

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Zika Virus (ZIKV) belongs to the Flavivirus genus, and the infections it causes, transmitted by Aedes species mosquitoes, pose an ongoing threat to public health. The positive-sense single-stranded RNA genome of Flaviviridae family members encodes for a single polyprotein co- and post-translationally cleaved by viral and cellular proteases, into structural and non-structural (NS) proteins [1]. Among the NS proteins, the highly conserved NS2B-NS3 protease, characterised by the catalytic triad – S135, H51, D75 – at the Nter region, is essential for the cleavage and the viral replication, representing a valid druggable target [2]. The structural homology between the active centre of NS3pro and various host serine proteases, as well as the inefficacy to date of peptide derivatives covalent inhibitors, make their development challenging. Thus, the identification of NS3proallosteric inhibitors appears as an alternative strategy, also considering that both vaccines and drugs are not commercially available. In our approach, in silico molecular dynamics, dynophore, virtual-screening, and synthetic methods, were applied with the aim to identify new potential hits.

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P.13 2-phenylquinoline derivatives activity on SARS-CoV-2 nsp13 helicase: insights from enzymatic activity and cell-based assays

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative agent of the Coronavirus Disease 19 (COVID-19) pandemic, leading to significant global morbidity and mortality. SARS-CoV-2 relies on a complex machinery for the replication and transcription of its genome. A crucial component of this machinery is the non-structural protein 13 (nsp13), which present a helicase activity. With multiple enzymatic functions, including unwinding and nucleoside phosphatase (NTPase) activities. Given its pivotal role in viral replication, nsp13 is a promising target for antiviral drug development. The inhibition of nsp13 helicase activity can disrupt the replication cycle of SARS-CoV-2 potentially leading to reduced viral load and amelioration of disease severity. In this study, we performed ATPase and unwinding assays to evaluate the activity of some 2-phenylquinoline derivatives designed and synthesized based on a previous hit, on SARS-CoV-2 nsp13. Results showed that some of them were able to inhibit both the nsp13 unwinding and NTPase activities with IC50 values in the micromolar range. Compounds were also tested on viral replication in SARS-CoV-2-infected VeroE6 cells. A few of them showed an inhibitory activity in the micromolar range and no cytotoxicity (CC50 > 100 uM).

P.14 Mapping the druggable antiviral targets in the replication cycle of mammarenaviruses

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Lassa fever, caused by the Lassa virus (LASV ; genus Mammaremavirus, family Arenaviridae, order Bunyavirales) is a deadly rodent-borne hemorrhagic disease that is endemic in West-African countries. Annually, an estimated 300.000 people become infected, resulting in up to 5.000 deaths but the impact of the virus extends well beyond these estimations. Beside LASV, at least seven other mammarenaviruses can be transmitted from rodents to human and cause severe disease (Lymphocytic choriomeningitis virus, Guanarito virus, Junin virus, Lujo virus, Machupo virus, Sabia virus and the Whitewater arroyo virus). Despite the recognized high epidemic and pandemic potential of mammarenaviruses, no specific treatments are available. Ribavirin is currently the only drug that has been approved for the treatment of LASV infections. It results in some antiviral effect when treatment is started early on after the first symptoms. The drug has a mostly a-specific and pleiotropic mechanism of antiviral action and treatment can result in severe anemia. Highly effective and specific antiviral drugs are therefore needed. There is, however, very little knowledge about the druggable targets in the replication cycle of mammarenaviruses. The viral polymerase and exonuclease as well as the entry and fusion processes are the best known targets, but many other undoubtedly exist. In order to lay the fundament to identify/map these druggable targets of mammarenaviruses replication cycle, we make use of the Mopeia virus (MOPV), which is closely related to LASV, even sharing the same rodent reservoir. MOPV is considered as a good a-pathogenic surrogate that can be handled safely under BSL-2 conditions. MOPV is nonpathogenic for non-human primates and has never been diagnosed in humans. We are currently developing and optimizing a dual-reporter antiviral assay suitable for high-throughput screening (HTS). Following the validation of this assay, we plan to initiate a HTS campaign of a 500k molecules diversity library from the Center for Drug Design and Discovery (CD3; www.cd3.be). In the meantime, we are developing a MOPV small-rodent infection model suitable for antiviral studies.

P.15 Proper Selection of In Vitro Cell Model Affects the Characterization of the Antiviral Neutralizing Antibody Response

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Antibody-mediated virus-neutralization involves the inhibition of viral infectivity by preventing viral entry into host cells. Neutralizing response is one of correlates of protection, contributing to long-term immunity against various viral infections. Numerous in vitro assays have been employed to evaluate neutralizing antibody activity, aiming to provide insights that enhance our understanding of these mechanisms in humans. The Vero E6 cell model has been widely used to study several viruses due to its dysregulation of the IFN response, ease of handling, and suitability for studying the basics of viral infections. In our study, we employed this in vitro model to assess whether antibodies evoked by first-generation smallpox vaccines can neutralize MPXV Clade II infection. Nevertheless, while Vero E6 cells are cost-effective and allow experiments to be conducted under controlled conditions, they do not recapitulate all cell-infection molecular events. As a proof of concept, our research underscores the importance of selecting appropriate cellular models to determine the neutralizing activity of antibodies against SARS-CoV-2 variants. Unlike the Vero E6 model, Calu-3 cells enabled the assessment of both viral entry pathways exploited by the virus. Our data revealed a lack of correlation between neutralization activity in Calu-3 and Vero E6 cells, suggesting that in vitro models permitting only one viral entry pathway may lead to misleading characterization of the neutralizing antibody activity depending on the SARS-CoV-2 variant tested. These findings highlight the critical need to choose suitable in vitro models for better assessing neutralizing activity of serum antibodies as well as novel entry inhibitors.

P.16 Enzymatic Preparation of Phospholipid Derivatives of Entecavir

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The arsenal against chronic hepatitis B (CHB) includes various antiviral agents, where nucleoside reverse transcriptase inhibitors play an important role. The most effective compound in this class, recommended by major health organizations, is entecavir, a guanosine nucleoside analogue. However, the use of entecavir has limitations, particularly in the treatment of CHB. It most often requires daily use throughout life, which reduces patient adherence to treatment and, as a result, negatively affects the effectiveness of treatment. One of the possible ways to overcome this drawback is the conjugation of entecavir with phospholipids and subsequent encapsulation in liposomes, which prolongs the action of the active compound and improves its pharmacological profile. In this study, entecavir was conjugated with phospholipids using the bacterial enzyme phospholipase D, which provides a one-step transphosphatidylation reaction. Dipalmitoylphosphatidylcholine and dihexanoylphosphatidylcholine served as phospholipid residue donors. As a result, entecavir samples with saturated diacyl phospholipid residues of 16:0 and 6:0 were obtained. The biotechnological method for preparing such compounds is easily scalable and suitable for synthesizing diverse phospholipid conjugates, which can be used to obtain new prodrugs based on nucleoside analogues with controlled drug compound release.

P.17 A Robust Mouse Model of Human Parainfluenza 3 Virus Infection to Study Prophylactic and Therapeutic Modalities

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Human parainfluenza virus type 3 (HPIV-3) can cause severe respiratory tract infections. The development of vaccines and antivirals is hampered by the lack of convenient small-animal infection models. Here, we show that intranasal inoculation of AG129 mice (double IFNα/β and IFNγ receptor knockout mice) with HPIV-3 leads to viral replication in the upper and lower airways, with peak viral loads on days 1-3 post-infection. By multiplex fluorescence RNAscope and immunohistochemistry followed by confocal microscopy, we demonstrate viral tropism to ciliated cells and club cells of the bronchiolar epithelium. From day 5 post-infection, a marked lung pathology develops, with perivascular and peribronchial inflammation, bronchopneumonia, and hyperplasia of pneumocytes. When HPIV-3-infected AG129 mice were co-housed with uninfected littermates, no transmission of the virus was observed. Oral treatment with GS-441524, the parent nucleoside of remdesivir, drastically reduces infectious virus titers in the lung, with a relatively normal histology. Intranasal treatment also affords an antiviral effect. We conclude that AG129 mice serve as a robust preclinical model for developing therapeutic and prophylactic strategies against HPIV-3. Our results support further evaluation of GS-441524 and its prodrug forms as potential treatments for HPIV-3 infection in humans.

P.18 Modulation of SARS-CoV-2 Nucleocapsid RNA binding by the host RNA helicase DDX3X

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The nucleocapsid protein Np of SARS-CoV-2 is involved in the replication, transcription, and packaging of the viral genome, but it also plays a role in the modulation of the host cell innate immunity and inflammation response. Ectopic expression of Np alone was able to induce significant changes in the proteome of human cells. The helicase DDX3X was found to physically interact with Np and to increase 2- to 4-fold its affinity for double-stranded RNA in a helicase-independent manner. Conversely, Np inhibited the RNA helicase activity of both protein. Afterward, the interaction of the individual Np-CTD and Np-NTD regions with DDX3X was evaluated, and at the same time of the regions DDX3X2-607, DDX3X132-607 and DDX3X132-662 with full-length Np. In the first case the interaction is greater between Np-ntd and ddx3x, in the second the DDX3X132-662 region has a good affinity with np.

P.19 Triazole-Based Compounds as Efficient Dimerization Disruptors of Leishmania infantum Trypanothione Reductas

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Although leishmaniasis is the second-largest deadly parasitic disease, only a limited number of drugs are available for the treatment. The search for new drugs against leishmaniasis, less toxic and acting by mechanisms of action different from those of the currently available drugs, is a global necessity. Trypanothione Reductase of Leishmania infantum (Li-TryR) is an essential and exclusive enzyme for the antioxidant defenses of these parasites, and is a validated target for the rational design of drugs against leishmaniasis. In our group we have developed an alternative inhibition strategy of the enzyme which consist on the disruption of the homodimeric interface of the Li-TryR.[1] The proof-of-concept of this approach was performed by using peptides and peptidomimetics that mimic 'hot spots' at the homodimeric interface. [2,3] In the search for new dimerization inhibitors with improved activity /toxicity profile, we have recently reported a symmetrical peptidomimetic based on 1,2,3-triazole-phenyl-thiazole scaffold.[4] By molecular modelling studies we identified, an almost unexplored hydrophobic region as putative binding site for this inhibitor located at the central interfacial domain of Li-TryR. In order to determine the potential as leishmanicidals and to carry out SAR studies we report here the design and synthesis of symmetrical triazole-phenyl-thiazole compounds of general structure modified at different positions.

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P.20 Development of a miniaturized high-performance FRET-based assay for screening of Nsp15 inhibitors

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Nonstructural protein 15 (Nsp15) encoded by coronavirus (CoV) is a uridylate-specific endoribonuclease that plays an essential role in the viral life cycle. Considering that Nsp15 is one of the major Interferon antagonists of SARS-CoV-2, it represents a critical component involved in viral pathogenesis. This makes it an interesting drug target to fight the recent pandemic and eventually future emergencies caused by this virus. To date, there are no approved Nsp15 inhibitors. The few active compounds have shown high IC50 and high cytotoxicity. Our goal is to identify novel small-molecule scaffolds targeting Nsp15 that are potent, bioactive, and nontoxic. To this aim, we developed a high-performance drug screening FRET-assay that allows us to study Nsp15 activity and test for potential inhibitors. First, Omicron Nsp15 recombinant protein was purified in two steps, performing an affinity chromatography followed by a size-exclusion chromatography. This resulted in obtaining a protein with 90% purity. Purified recombinant Nsp15 was tested for its ability to cleave the substrate – a ssRNA with a fluorophore attached to the 5' end and a quencher attached to the 3' end, containing a U cleavage site in the middle. When the enzyme cleaves the substrate, a fluorescent signal is released. All assay parameters were optimized. Enzyme (30 nM), substrate (1.5 mM), and Mg²⁺ (5 mM) concentrations were determined. Presence of reducing agents, pH, and BSA addition were also optimized. After optimization, the z-factor was calculated and resulted in a value between 0.7 - 0.9, confirming the robust assay performance.

P.21 Antiviral properties exerted by Pistachio extracts and zeaxanthin against SARS-CoV-2 variants in vitro binding models

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Introduction: Since the pandemic's onset, SARS-CoV-2 variants have posed challenges despite antiviral drug use, leading to exploring plant-derived products for their antiviral potential.Aim: This study aimed to evaluate the potential antiviral activity of pistachio extracts (NRRE and RURE) and zeaxanthin, known for their antiviral activity against HSV-1 [1], against SARS-CoV-2 by assessing their ability to inhibit the binding of pseudotyped viral particles. Materials and Methods: The pseudotyped particles bearing the S protein of alpha (α) and omicron (ο) variants of SARS-CoV-2 were constructed by using a murine leukemia virus (MLV)-based packaging system and characterized by the luciferase assay [2]. The binding inhibition assay was performed on A549 cells transduced with α and ο- SARS-CoV-2 pseudoviruses previously treated with 0.6mg/mL of NRRE and RURE and 10 μM of zeaxanthin. TMPRSS2 expression was analyzed via western blot. Results: NRRE and RURE inhibit α- and ο-SARS-CoV-2 pseudovirus binding, while zeaxanthin inhibits α-SARS-CoV-2 pseudovirus internalization, most likely, by targeting TMPRSS2.Conclusion: The findings identify new potential natural compounds in pistachio extracts and zeaxanthin for protecting human cells from SARS-CoV-2 infection.

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P.22 Determining the proteolytic fingerprint of Lake Geneva to investigate the mechanism of virus inactivation in lake water

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Many human viruses are transmitted via the environment and thus come into contact with indigenous microorganisms. Bacteria are known to decrease the persistence of viruses in the environment through the action of their secreted extracellular enzymes. Indeed, Echovirus 11 (E11) and Human Adenovirus type 2 (HAdV2) show significant inactivation in biologically active Lake Geneva water. However, the effect of entire microbial communities on virus persistence is poorly understood. To provide new insight into the mechanisms of virus inactivation by microbial communities, Multiplex Substrate Profiling by Mass Spectrometry (MSP-MS)[1]was used to characterize the proteolytic activity of the entire pool of microbial enzymes in Lake Geneva water. This analytical method, based on a library of 124 synthetic tetradecapeptides acting as model substrates, generates protease specificity profiles. The observed cleavage patterns can then be linked to viral capsid sequences to predict viral inactivation. Our results reveal a preference for proteolytic cleavage after positively charged (arginine and lysine) and aromatic (tyrosine and tryptophan) amino acids, while negatively charged residues (glutamic acid and aspartic acid) are disfavored by the proteases present in lake water. The proteolytic specificity profiles can be mapped to surface-exposed motifs on viral capsid proteins to identify possible cleavage sites, providing insight into the mechanism of viral inactivation by microbial proteases. Thus far, 5 possible cleavage sites were identified on the surface of E11 and 2 were identified on the fiber protein of HAdV2. These results will deepen our understanding of the relationship between viral environmental persistence and enzyme production, which is crucial for maintaining safe drinking and recreational water as well as optimizing biological sewage treatment methods for the protection of public health.

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P.23 In vitro cytotoxicity and anti-influenza activity of silver nanoparticles loaded in various polymer drug delivery systems for topical administration

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Influenza is an acute respiratory infection with high morbidity, pandemic potential and drug-resistance rates. Silver nanoparticles (SN) provide an alternative therapeutic approach as they have high yields, solubility and stability and have shown antiviral properties based on literature data. Cytotoxicity, antiviral and virucidal effect of 250 μg/ml SN alone and loaded in 1% polymers for topical use in pharmaceutical development – PVP, PEG 4000, MC, CMC, HPMC, C940, ES, EL was studied in MDCK cell line, using A/Panama (H3N2) and B/Lee influenza strains. Light microscopy and Neutral Red uptake assay were used for CC50, IC50, and selectivity index (SI) calculation. Maximum tolerable concentrations (MTC) were used to evaluate the virucidal activity after incubation of samples with a virus 15, 30 and 60 min intervals as differences in CPE, as Δlg, were recorded between the treated groups and virus control. CC50 of SN administered alone in MDCK cells was 72,69 μg/ml. When loaded in 7 studied polymers CC50 varied between 75.01 μg/ml (C940) and 154,52 μg/ml (ES) which suggest that delivery systems do not increase but even diminish toxic effects in vitro. An exception was C940 - 36.81 μg/ml likely originating from the polymer's viscosity. SN had a weak effect on H3N2 virus replication, with IC50 = 37.21 μg/mL and SI = 1.95. SN alone did not exert protection against B/Lee strain but mixed with MC, CMC and PG4000 SI varied between 1.68 and 3.23. Virucidal activity test showed dependency on the incubation time from 15 to 60 min for all samples with SN with highest Δg of 4.0 lg at 60 min for nanoparticles dissolved in C940 and MC. Our data suggest that SN, mostly acting as virucidal agents, might be included in all the studied polymers for development of topical drugs for influenza treatment.

P.24 Role of cyclooxygenases 1 and 2 in herpes simplex type 1-infected dendritic cells

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Herpes simplex virus type 1 (HSV-1) is a prevalent microorganism that produces lifelong infections in humans by establishing latency in sensory and autonomic neurons. HSV-1 also infects dendritic cells (DCs), which are professional antigen-presenting cells that initiate and regulate antiviral immune responses. Importantly, HSV-1 negatively modulates DC function and ultimately kills these cells. Cyclooxygenases (COXs) are host enzymes that metabolize arachidonic acid into prostaglandin G2 (PGG2), which is subsequently converted into prostaglandin H2 (PGH2). PGH2 acts as a precursor of PGE2, PGD2, and PGI2 synthesis, as well as thromboxane (TXA2), which are involved in inflammatory and non-inflammatory processes. Previous reports indicate that COX-2 products can suppress the functions of DCs. Here, we explored the role of COXs in the modulation of DC function after infection with HSV-1. We found that HSV-1 infection significantly modulates the expression of COX-2 in infected DCs, as determined by RT-qPCR and Western Blot, and that the pharmacological inhibition of COX-2 recovers the viability of DCs infected with this virus, modulates their cytokine profile and maturation, altogether promoting T cell activation. Interestingly, the pharmacological inhibition of COX-2 did not impact the yield of HSV-1 virions from DCs. These results indicate that HSV-1 induces COX-2 expression in DCs, which relates to the death of these cells. Authors are supported by the Millennium Institute on Immunology and Immunotherapy #ICN2021_045.

P.25 Optimization of high-sensitivity assay for HTS of potential inhibitors against WNV NS2B-NS3 protease.

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West Nile virus (WNV) is a human pathogen belonging to the Flaviviridae family, responsible of widespread disease for which there is no vaccine currently available. One attractive target for antiviral development is the viral trypsin-like serine protease NS2B-NS3, a heterodimeric complex between the hydrophilic domain of the cofactor, NS2B (NS2BH) and the protease domain (NS3-pro). During viral replication, the NS2B-NS3 protease, together with host proteases is responsible for the cleavage of the flavivirus polyproteins, leading to active viral proteins. Despite strong efforts in the development of antivirals due to its essential role in the viral replication, no protease inhibitors have reached clinical trials yet. We expressed and purified the viral protease, obtaining a high-purity product and optimized a FRET-based fluorescent assays, employing a labelled peptide substrate. We determined the optimal assay conditions, such as reaction mix composition, enzyme and substrate concentration and optimal incubation time, obtaining an assay with high sensitivity and specificity, useful for the enzymatic characterization and high-throughput quantitative screening (HTS) of potential inhibitors. The optimized assay proved to be a rapid, reproducible tool with a robust Z factor, for screening potential inhibitors against the WNV NS2B-NS3 viral protease. The validity of the assay was demonstrated by confirming the inhibitory activity of aprotinin, used as a positive control given its known ability to inhibit the WNV NS2B-NS3 protease. Screening of compounds given by our collaborators is on-going.

P.26 Integrating CADD and synthetic methods to identify novel pan-viral helicase inhibitors

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The study uses the Helicase-targeting Small Molecule Antiviral Compound Collection (Heli-SMACC)1 to identify compounds active against superfamilies 1-3 (SFs) viral helicases.2 A KNIME workflow was applied to select the best and most diverse inhibitors. Synthetic procedures were established to synthesize reference compounds, which will be repurposed for biological testing on other helicases and activity validation. The results will be used for similarity screening, scaffold validation, and the design of synthesizable libraries to enhance activity.

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P.27 DDX5 an emerging treater for SARS-CoV-2 infection

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DDX5 is a member of the DEAD box family of RNA helicases that is involved in different cellular processes as a result of its role as an adaptor molecule, promoting interactions with a large number of other factors. This protein is involved in pathways that include the remodelling of RNA structures, regulation of transcription and the processing of small noncoding RNAs. DDX5 deregulation is associated with tumors, in particular lung cancer, but it also plays a role in viral replication, including replication of the SARS-CoV-2 genome. In our previous study we showed, for the first time, a direct interaction of SARS-CoV-2 Nucleoprotein (Np) with the host DDX1 and DDX3X helicases and their reciprocal effects on RNA interaction. Then we started to analyse DDX5. The expression and purification of recombinant SARS-CoV-2 Np and human DDX5 confirmed their direct physical interaction. Biochemical characterization of these proteins, either alone or in combination, showed that they reciprocally modulated their interaction with dsRNA. For this reason, we studied protein–protein interaction inhibitors that could be designed to disrupt the binding of Np to DDX5. We tested 30 DDX5 inhibitors and we found two interesting compound SI_FM 201 and 202. These compounds perturbed DDX5 activity in ATPase and helicase assay, both on dsRNA and R-loops structures. These findings can contribute to identify new mechanisms in viral infection and novel therapeutic approaches.

P.28 Repurposing AKt inhibitors for the treatment of Orthoflavivirus infection

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Zika and Usutu are arbovirus transmitted mainly by mosquitoes to humans. Zika virus is of great health concern, while Usutu virus is a potential dangerous and emerging risk for the near future. In addition, both are currently spreading widely throughout the world. For this reason, strategies to prevent its transmission and replication are a priority research topic.
Among the different host pathways controlled by these viruses, the PI3K/Akt/mTOR is dysregulated during flavivirus infection. We demonstrated for the first time the interaction between the cellular kinase Akt and ZIKV NS5 during ZIKV infection in different cell lines (mammalian and mosquito). We studied the effect of two Akt inhibitors widely used, Miransertib (ARQ-092, non-competitive inhibitor) and Capivasertib (AZD5363, competitive inhibitor) on Zika and Usutu virus replication in these cell lines. Miransertib showed a stronger inhibition of ZIKV and USUV virus than Capivasertib in mammalian cells. However, the competitive inhibitor Capivasertib showed a stronger effect in mosquito cells. These results demonstrate that Akt plays an important role during flavivirus infection independently of the cellular model employed, although its role could be different in cells from different origins. Furthermore, the results obtained with these molecules, which are currently used in clinical trials in different types of cancer, opens a new insight in the inhibition of flavivirus replication.

P.29 Development of hybrid hydroxychloroquine liposomes with Ag-nanoparticles as pan-HCoVs antivirals.

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Viruses affect millions of people worldwide, resulting in a significant impact on human health and socioeconomic development. The COVID-19 pandemic's devastating impact has driven the development of safe and effective antivirals to decrease the risk of infection and mortality. In the rush towards the development of novel therapeutic strategies liposomes are being viewed as a promising delivery method because of their compatibility with drugs, vaccines, and other therapeutic molecules. Exploiting the well-known antiviral properties of silver (Ag) and hydroxychloroquine (HCQ), four different sets of nanoscale liposomes were developed: empty liposomal dispersion (E-Lipo) was combined with HCQ (HCQ-lipo), Ag (Ag-lipo) or both HCQ and Ag (Combi-lipo), aiming to increase HCQ and Ag antiviral effect and reduce cell mortality. The formulations were tested for their cellular compatibility and to evaluate the ability to inhibit HCoV-229E, MERS-CoV and SARS-CoV-2 replication in different cellular systems. BEAS-2B cells were infected with HCoV-229E for 72h, while Vero E6 GFP cells were infected with SARS-CoV-2 for 72h and MERS-CoV for 96h. The overall results showed a reduction in EC50 levels in all the systems tested for both HCQ-lipo and Combi-lipo as compared to the HCQ alone, while the cytotoxicity lightly increased for both HCQ-lipo and Combi-lipo with respect to the empty formulation or the HCQ alone, possibly as consequence of a higher HCQ cellular availability and cytotoxicity. Unexpectedly the Ag-lipo didn't show any antiviral effect in the system tested, not even improved the antiviral effect of HCQ in the Combi-lipo formulation as compared to the HCQ-lipo. As expected, the E-lipo didn't show any antiviral effect.

P.30 Differential expression of Human Endogenous Retroviruses in Chronic and Acute Myeloid Leukemia at Diagnosis and after TKI Therapy

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Human Endogenous Retroviruses (HERVs) represent the ~8% of the human genome. They are involved in physiological functions and able to both modulate and be influenced by the host immune system. Despite this could likely led to pathological manifestation, especially in cancer where HERV tend to be transcriptionally de-repressed, their role in pathological contexts is still poorly characterized. The aim of this study was to evaluate HERV transcriptome in patients affected by two hematologic malignancies, Chronic Myeloid Leukemia (CML) and Acute Myeloid Leukemia (AML), to explore their possible link to pathogenesis. The population was composed by 8 HC (healthy controls) and 25 patients, of which 7 were affected by AML and 17 in the remission phase after CML therapy with TKI. For 5 of the CML patients, also samples before TKI treatment were considered. HERV transcriptome resulted significantly modulated in patients, clearly dividing them from HC. In addition, specific signatures of HERV modulation divide CML and AML patients as well as actively affected patients with CML from the ones in remission. A total of 389 HERVs were significantly modulated in leukemia patients vs HC. Moreover, 320, 389, and 177 modulated HERVs were found in the individual analysis of AML patients vs HC, CML patients vs HC, and CML patients in remission vs HC, respectively. Finally, 9 common HERVs were differentially expressed in all the conditions considered in the study. These identified HERVs will be further tested for their pathogenic potential and diagnostic value for AML and CML.

P.31 Novel approach for the design of universal recombinant vaccines to control diseases caused by viruses with high antigenic variability

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There are a number of virus infections that are caused by viruses with high antigenic variability. This could provide obstacles for preventing these diseases by vaccination because of the discrepancy of antigen sequence within vaccine and circulating virus strains. Protein subunit vaccines are characterized with the best safety profile, simplicity of production and are a promising tool for the development of wide-spectrum vaccines. In the present study two approaches were applied to design synthetic antigens — the application of a highly conserved neutralizing epitopes and the use of the consensus sequences. Universal recombinant rotavirus A antigen – URRA, Newcastle disease virus (NDV) antigen – Castle1 and a panel of COVID-19 antigens were produced. Coronavirus antigens effectively interacted with sera from donors recovered from different COVID-19 variants. For the URRA the recognition by antisera to patient-derived field rotavirus isolates were proven. And antibodies specific to Castle1 were revealed to recognize field NDV isolates. The often problem in developing subunit vaccines is low immunogenicity of individual recombinant proteins. Here, we used a unique protein adjuvant – spherical particles (SPs), which is structurally modified plant virus. SPs effectively improved the production of antibodies specific to the URRA or coronavirus antigens within corresponding vaccine composition. COVID-19 vaccine candidate was shown to induce antibodies, which bound recombinant S-protein of Omicron variant. It provides evidence that the development of subunit protein vaccines with the application of synthetic biology approach is a promising strategy for highly cross-reactive vaccine creation.

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P.32 Probing Potential Pan-hCoV Inhibitors by Targeting Nsp13 Helicase

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Given its highly conserved sequence and essential function in viral replication, the helicase of human coronaviruses (hCoVs) represents a compelling and promising target for drug development. Targeting this enzyme could potentially lead to treatments for various hCoVs, which cause a range of conditions from mild, self-limiting respiratory infections to severe, life-threatening diseases. [1] In this work, with the aim of identifying new potential Pan-hCoV helicase inhibitors, crystallographic structures of SARS-CoV-2 helicase available to date [2] have been considered to rationally design a library of about 100 compounds that have been subsequently synthesized and characterized by structural (single-crystal X-ray diffraction) and spectroscopic (NMR, MS) methods. All compounds were found to inhibit both SARS-CoV-2 helicase-associated enzyme activities, namely NTPase and unwinding activity, showing IC50 values in the low micromolar range; among them, several compounds inhibited SARS-CoV-2 replication with low EC50 and no significant CC50 values. In addition, some of the most potent compounds exhibited pronounced antiviral activity against HCoV229E and MERS-CoV, highlighting some of them as promising Pan-hCoV helicase inhibitors. These findings indicate that the hCoV helicase is a viable target for developing new drugs to treat infections caused by SARS-CoV-2 and other human coronaviruses. Such drugs could be instrumental in addressing future emerging and re-emerging infectious coronavirus diseases.

1.Spratt, A.N., et al., 2021. 31(4): p. 339-350.
2.Newman, J.A., et al., 2021. 12(1): p. 4848.
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P.33 Mucroporin-M1 analogues: synthesis, conformation and structure-activity correlation

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Mucroporin is a peptide of 17 amino acids deriving from the scorpion venom Lychas mucronatus with known antimicrobial properties (active against gram-positive bacteria). "Li et al." optimized the Mucroporin sequence by proposing Mucroporin-M1 sequence with some amino acid substitutions (G3R, P6K, G10K and G11R). These modifications improved peptide activity at lower concentrations respect to the native peptide. Substitutions increase the net positive charge of the peptide, strengthening the amphipathic character of the peptide helix. From previous studies, it was seen that Mucroporin-M1 interacts directly with the viral particles and upon attachment of the peptide to the virus, its strong electrostatic affinity enhances the interaction and destruction of the virus envelope[1-3]. The purpose of our work was to further improve the activity of Mucroporin-M1 by appropriately modifying the amino acid sequence and studying the conformation and the structure-activity relationship. We modified the sequence by inserting the non-proteinogenic amino acid α-aminoisobutyric acid, Aib (Aib5I, Aib9I and Aib13V) in order to strengthen the helical conformation and to improve the stability towards proteolytic enzymes. We also synthesized some shortened Mucroporin-M1 analogues to identify the shortest active sequence. This will allow us to develop a convenient and easy synthetic protocol. All peptides were fully characterized and their conformation was studied by NMR and circular dichroism. We report an investigation on the stability of the Mucroporin-M1 analogues to proteolytic enzymes and on the propensity of the peptides to interact with biological membranes. Preliminary results on the antimicrobial and antiviral properties of the synthesized peptides were reported, and a correlation between biological activity and 3D structure was discussed.

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P.34 Targeting West Nile virus replicases: NS3 and heterodimers inhibitors

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The West Nile virus (WNV) is a flavivirus of the Flaviviridae family. It circulates through a mosquito–bird–mosquito cycle and is transmitted to humans through Culex mosquitoes. In approximately 1 in 150 infections, WNV results in encephalitis or meningitis. The actual pharmacotherapy of WNV includes only the treatment of symptoms. Alongside this, the flaviviral evolutionary process, drug resistance, and the absence of vaccination strategies evoke urgency for the development of new antiviral compounds. Non-Structural protein 3 (NS3) of WNV represents a suitable starting point for a screening campaign aiming at the identification of new antiviral drugs. The work aims to identify several peptides or peptidomimetics that could act as antiviral to inhibit WNV NS3 activity by different approaches. Firstly, a direct approach targeting the active site of NS2B-NS3 protease exploiting peptides and peptidomimetics as potential inhibitors; secondly, an indirect approach targeting protein-protein interactions (PPi) that are essential for NS3 activity, specifically those involved in the formation of NS2B-NS3 heterodimers. We designed and studied by docking some peptides and peptidomimetics that could act as inhibitors against the NS2B-NS3 protease of West Nile virus. The most promising compounds were synthesised and tested, and their efficacy and activity against protease evaluated. The results were then analysed and used to design new peptide structures to improve their inhibitory activity.


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