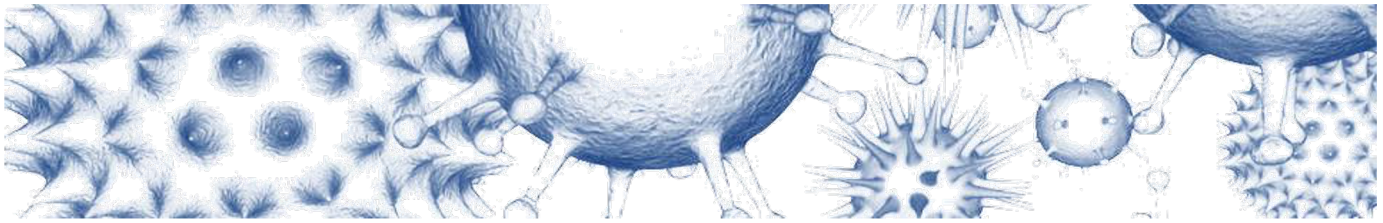
5th Innovative Approaches for Identification of Antiviral Agents Summer School

September 22nd-24th 2021

Virtual meeting

Program & Abstract Book



Dear Participant

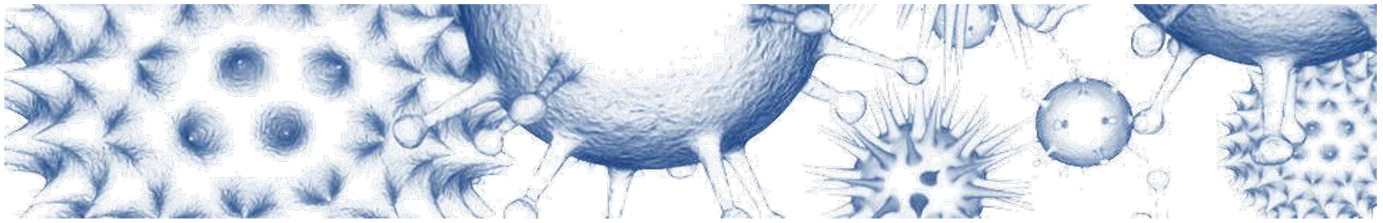
Following the success of the previous editions, we have the pleasure to welcome you to the "Innovative approaches for the identification of antiviral agents" summer school, with the patronage of Regione Autonoma della Sardegna, University of Cagliari, University of Minnesota, Sardegna Ricerche Research agency, 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 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!

The Summer School Organizing Committee



Organizing Committee

Enzo Tramontano
Department of Life and Environmental Sciences
Laboratory of Molecular Virology
SS554, Cittadella di Monserrato
09042, Monserrato (Cagliari), Sardinia, Italy
e-mail: tramon@unica.it

Reuben Harris
HHMI and University of Minnesota
Minneapolis, Minnesota, USA, 55455
e-mail: rsh@umn.edu

Angela Corona
Department of Life and Environmental Sciences Laboratory of Molecular Virology
SS554, Cittadella di Monserrato
09042, Monserrato (Cagliari), Sardinia, Italy
E-mail: angela.corona@unica.it

Elias Maccioni
Department of Life and Environmental Sciences
Laboratory of Drug Synthesis
Palazzo delle Scienze
09125, Cagliari, Sardinia, Italy
e-mail: maccione@unica.it

Cristina Parolin
Department of Molecular Medicine
University of Padua
Via Gabelli 63, 35121, Padua, Italy
e-mail: cristina.parolin@unipd.it

Stuart F. J. Le Grice
Reverse Transcriptase Biochemistry Section HIV
Drug Resistance Program
National Cancer Institute – Frederick
Frederick MD 21702, USA
E-mail: legrices@mail.nih.gov

COSMESE
Consorzio per lo Studio dei Metaboliti Secondari

Secretariat:

Dr. Maria Paola Pisano
Phone +39 070 675-4535/4533
Fax +39 070 675-4536 email: iaaass@unica.it

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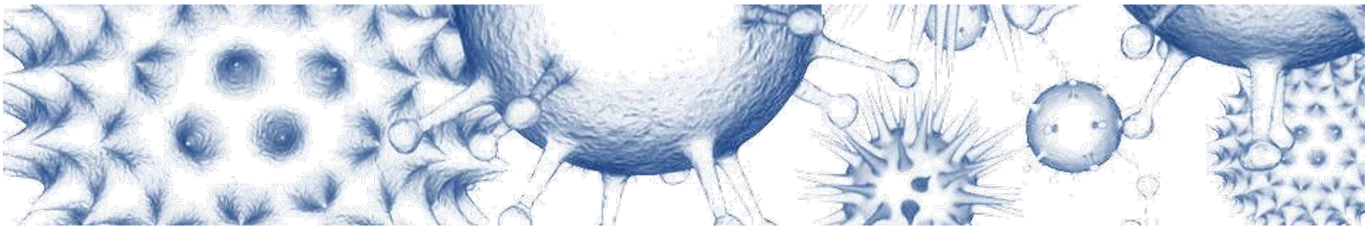
International Antiviral Symposium Foundation


Innovative Approaches for the identification of Antiviral Agents Summer School
5th IAAASS
22-24/09/2021

Program



WEDNESDAY, SEPTEMBER 22

ALL TIMES ARE LOCAL (SARDINIA STANDARD TIME)

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5 – 5:10 PM **Opening Remarks**

Convener: **Enzo Tramontano,** University of Cagliari, Italy
Reuben Harris, HHMI and University of Minnesota, United States

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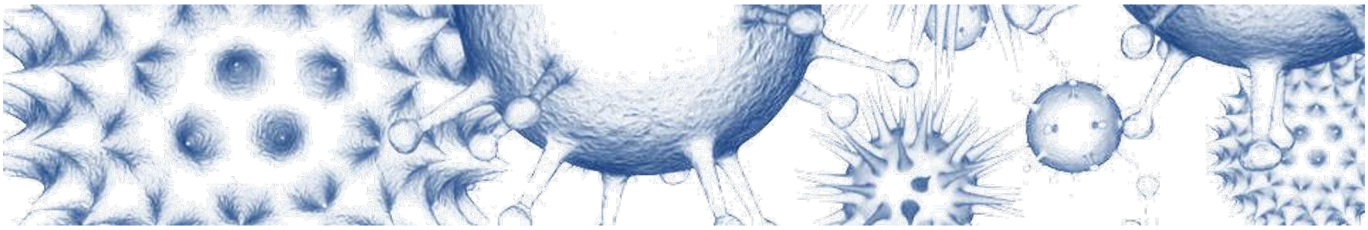
5:10 – 6:00 PM **Opening Plenary**

1 – **Akiko Iwasaki**, Yale School of Medicine, United States “Immune responses to SARS-CoV-2”



THURSDAY, SEPTEMBER 23
ALL TIMES ARE LOCAL (C.E. TIME)

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9:55 – 10:00 AM **Opening Remarks**

Convener: **Reuben Harris,** HHMI and University of Minnesota, United States

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10:00 – 11:00 AM **Breakout Session 1 - Individual Breakout Rooms 1-13**

2 – **Low Zhao Xuan**, Sunway University, Malaysia “Antiviral activity of silymarin, baicalein and baicalin against Dengue virus”

3 – **Nicolò Santi**, Cardiff University, United Kingdom “Identification of novel non-nucleoside inhibitors of Dengue virus NS5 RNA-dependent RNA polymerase”

4 – **Saverio Lemme**, MIMIT – Tor Vergata, Italy “An application of the obtained comparison results from the analysis of three classes of patients pharmacologically treated and untreated to the structure of the HIV-1 integrase to obtain a deeper understanding of the observed mutations”

5 – **Saumya Singh**, Sam Higginbottom University of Agriculture, Technology & Sciences, India “Design and development of novel oxadiazole based 1,3,5-Triazines as NNRTI targeting entrance channel of NNRTI binding site ”

6 – **Emmanuel Heilmann**, Medical University Innsbruck, Austria “Cell-based cis-cleavage screening method for SARS-CoV-2 protease inhibitors”

7 – **Francesca Miglioli**, University of Parma, Italy “Synthesis of polyhydroxylated heterocyclic compounds as inhibitors of Influenza virus pan endonuclease”

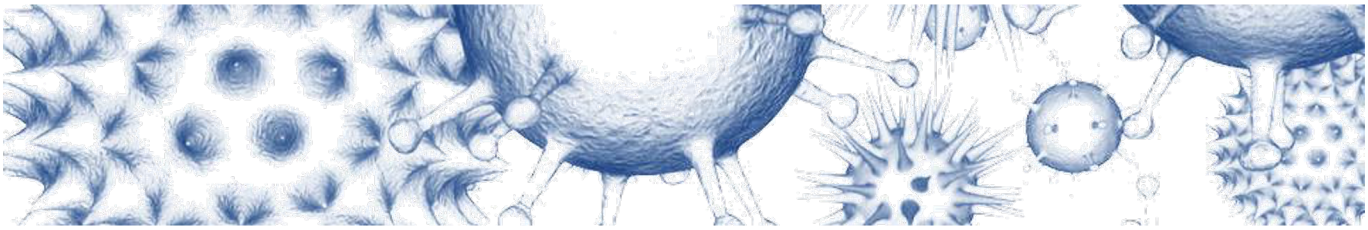
8 – **Dominik Werz**, University of Hambur, Germany “Lipophilically, bioreversibly masked cyclic nucleoside monophosphate derivatives - Tools for non-invasive cell assays”

9 – **Ekaterina Ryabchevskaya**, Lomonosov Moscow State University, Russian Federation “The development of broad-spectrum recombinant Betacoronavirus vaccine with plant virus-based platform-adjuvant”

10 – **Annalaura Paulis**, University of Cagliari, Italy “Flavonoids induce type I Interferon expression through STING dependent pathway”

11 – **Annalaura Brai**, University of Siena, Italy “Exploring the implication of DDX3X in DENV infection: discovery of the first-in-class DDX3X fluorescent inhibitor”

12 – **Lia-Raluca Olari**, Ulm University, Germany “An amyloidogenic fragment of human alpha hemoglobin with a combined antibacterial and antiviral activity”



13 – **Selina Pasquero**, University of Turin, Italy “Novel antiviral activity of PADs inhibitors against Beta-coronaviruses SARS-CoV-2 and HCoV-OC43”

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Main Session 1:

Convener:	**Cristina Parolin**, University of Padua, Italy
11:00 – 11:10 AM	Overview of the session
11:10 – 11:40 AM	14 – **Graciela Andrei**, KU Leuven, Belgium “Herpesvirus DNA polymerases and impact of mutator phenotypes”
11:40 – 12:10 AM	15 – **Matteo Iannacone**, Vita-Salute San Raffaele University, Italy “Controlled administration of aerosolized SARS-CoV-2 to K18-hACE2 transgenic mice uncouples respiratory infection and anosmia from fatal neuroinvasion”
12:10 – 12:40 AM	16 – **Ali Mirazimi,** Karolinska Institutet, Sweden “Using recombinant human Angiotensin-Converting Enzyme 2 (rhACE-2) as antiviral against SARS-CoV 2”
12:40 AM – 01:10 PM	17 – **Roberto Di Santo**, Università di Roma La Sapienza, Italy “Design of selective HIV-1 RNase H inhibitors starting form DKA dual inhibitors of IN and RNase H”
01:10 – 01:40 PM	18 – **Andrea Brancale**, Cardiff University, United Kingdom “ Structure-based drug design in antiviral drug discovery”

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01:40 – 03:00 PM	**Long break (breakout rooms 2-18 open for discussion)**

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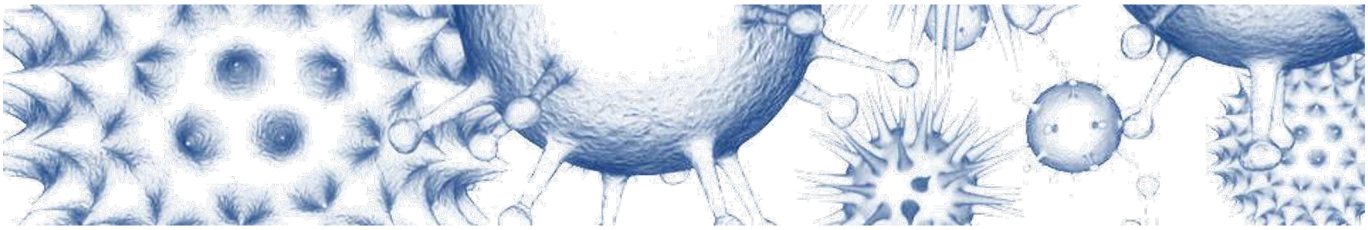
Main Session 2:

Convener:	**Angela Corona,** University of Cagliari, Italy
03:00 – 03:10 PM	Overview of the session
03:10 – 03:40 PM	19 – **Jason McLellan**, University of Texas Austin, United States “Structural approaches to SARS-CoV-2 therapy and strategies to address the inevitability of resistance”
03:40 – 04:10 PM	20 – **Reuben Harris**, HHMI and University of Minnesota, United States “Innovative herpesviral mechanisms to escape restriction by cellular APOBEC enzymes”



04:10 – 04:40 PM 21 – **Katherine Seley-Radtke**, University of Maryland, United States "Rational (and sometimes irrational!) strategies in nucleoside drug design"

04:40 – 05:10 PM 22 – **St. Patrick Reid**, Omaha University, United States "Elucidating the pathogenic mechanisms of chikungunya virus"

05:10 – 05:40 PM 23 – **Peter Stockley**, University of Leeds, United Kingdom "Structural & functional investigations of a murderous viral enzyme in a native-like context"

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05:40 – 06:00 PM **Short break (breakout rooms 19-23 open for discussion)**

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06:00 – 07:00 PM **Breakout Session 2 - Individual Breakout Rooms 24-35**

24 – **Alessia Onali**, University of Cagliari, Italy "Inhibitors of HIV-1 RT: exploring new scaffolds for the dual inhibitionof polymerase and ribonuclease associated functions"

25 – **Charles Waters**, University of Maryland, United States "Synthesis of flexible nucleos(t)ide analogues of remdesivir in broad specturm antiviral therapeutics"

26 – **Elisa Fanunza**,University of Minnesota, United States "Human cytomegalovirus mediates APOBEC3B relocalization early during infection through a ribonucleotide reductase-independent mechanism"

27 – **Luisa Fernanda Duarte Peñaloza**, Pontificia Universidad Católica Chile, Chile "Characterization of vaccine breakthrough cases exhibited in a cohort of Chilean adults immunized with an inactivated SARS-CoV-2 vaccine"

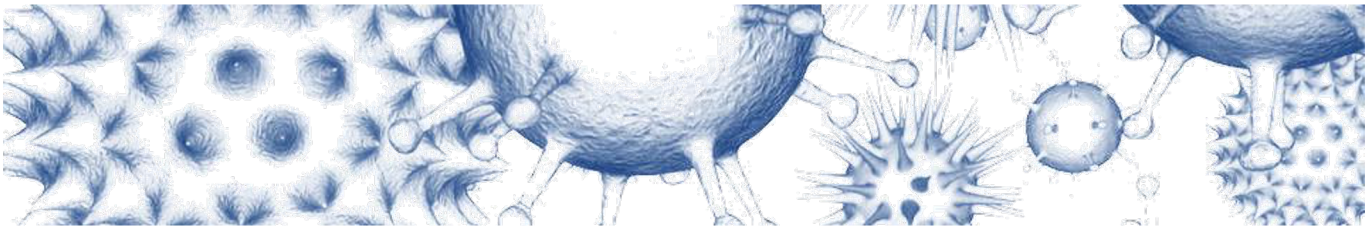
28 – **Nathan Ponzar**, Saint Louis University, United States "Complex inhibition characteristics of HBV and HIV RibonucleasE H inhibitors against human ribonuclease H1"

29 – **Sante Scognamiglio**, University of Cagliari, Italy "Identification, comprehensive characterization and comparative genomics of the HERV-K(HML8) integrations in the human genome"

30 – **Tyng Hwey Tan**, University of Aberdeen, United Kingdom "Monoclonal human antibodies that recognise the exposed N and C terminal regions of the often-overlooked SARS-COV-2 ORF3a transmembrane protein"

31 – **Seyad Arad Moghadasi**, University of Minnesota, United States "Gain-of-function assay for SARS-CoV-2 Mpro inhibition in living cells"

32 – **Desiree Rijono**, University of Hamburg, Germany "Optimization of a phenylethynyl anthranilic acid-based dihydroorotate dehydrogenase inhibitor series for the use as antiviral agent"



33 – **Benedikt Ganter**, University of Hamburg, Germany "Thesis of modified T-1106-5´-triphosphates as potential inhibitors of the SARS-CoV-2 RNA polymerase"

34 – **Joy E. Thames**, University of Maryland, United States "Design, synthesis, and biological evaluation of flex-acyclovir analogues against SARS-CoV-2"

35 – **Oriana Tabarrini**, Universiy of Perugia, Italy "Exploiting a proprietary compound library to fight SARS-COV-2: identification of 2-phenylquinolines with pan-anticoronavirus activity"

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07:00 – 09:00 PM **Refreshments (breakout rooms 2-35 open for discussion)**

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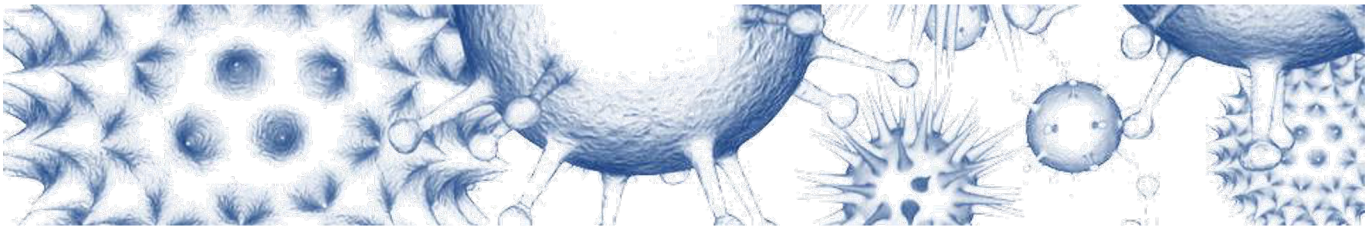

FRIDAY, SEPTEMBER 24

ALL TIMES ARE LOCAL (C.E. STANDARD TIME)

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11:55 – 12:00 AM **Opening Remarks**

Convener: **Enzo Tramontano,** University of Cagliari, Italy

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Main Session 3:

Convener: **Elias Maccioni**, University of Cagliari, Italy

12:00 – 12:10 AM Overview of the session

12:10 – 12:40 AM 36 – **Giorgio Gribaudo**, University of Torino, Italy "The US12 protein family of the Human Cytomegalovirus: from functions to antiviral intervention"

12:40 AM – 01:10 PM 37 – **Branka Horvat**, International Center for Infectiology Research-CIRI, France "Development of fusion inhibitory peptides against airborne viral infections, measles and COVID-19"

01:10 – 01:40 PM 38 – **Luis Menendez- Arias**, Centro de Biología Molecular Severo Ochoa, Madrid, Spain "HIV-1 replication: fidelity, drug resistance and unexploited antiviral targets"

01:40 – 02:10 PM 39 – **Vincenzo Summa**, Federico II University, Italy "A journey from HCV protease to SARS-CoV-2- 3Clp protease inhibitors in clinical trial: challenges and opportunities"

02:10 – 02:40 PM 40 – **Johan Neyts**, KU Leuven, Belgium "Antivirals, a lot has been achieved, yet a long way to go"

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02:40 –04:00 PM **Long break (breakout rooms 36-40 open for discussion)**

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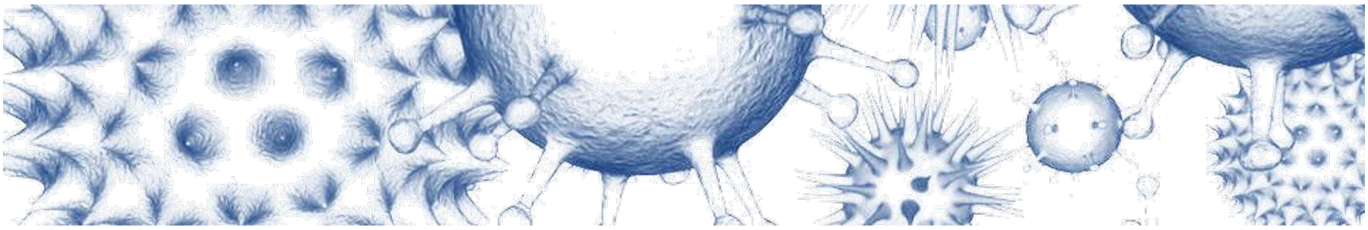
Main Session 4:

Convener: **Reuben Harris**, HHMI and University of Minnesota, United States

04:00 – 04:10 PM Overview of the session

04:10 – 04:40 PM 41 – **Anne Simon**, University of Maryland, United States "Developing novel virus-like agents to combat viral, bacterial, and other pathogens of trees to prevent a future without citrus, chocolate, grapevines and olives"



04:40 – 05:10 PM	42 – **Yun-Xing Wang**, National Cancer Institute, United States "Direct visualization of individual RNA molecules and mapping the RNA conformational space in solution"
05:10 – 05:40 PM	43 – **Josè Estè Araque**, UIC, Spain "Identifying new antivirals, resistance and host-factors"
05:40 – 06:10 PM	44 – **Michelle Hastings**, Rosalind Franklin University, United States "Antisense oligonucleotides for the control of gene expression in disease and infection"
06:10 – 06:40 PM	45 – **Luca Guidotti**, Vita-Salute San Raffaele University, Italy "Animal models for the development of antiviral agents against hepatitis B virus (HBV)"

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06:40 – 08:00 PM **Refreshments (breakout rooms 36-45 open for discussion)**

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Innovative Approaches for the identification of Antiviral Agents Summer School
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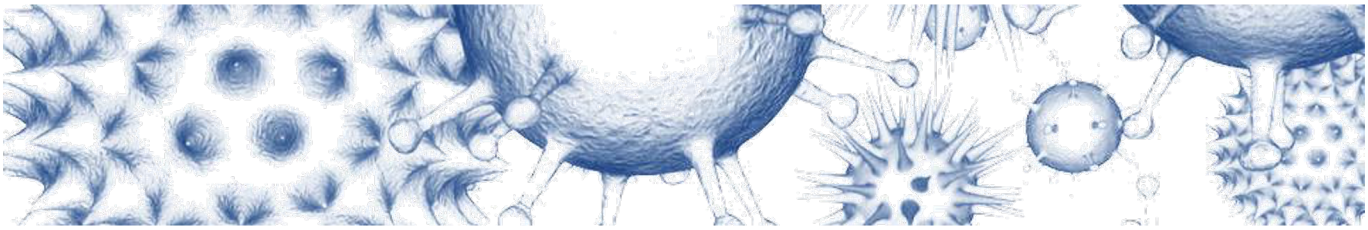
Abstracts

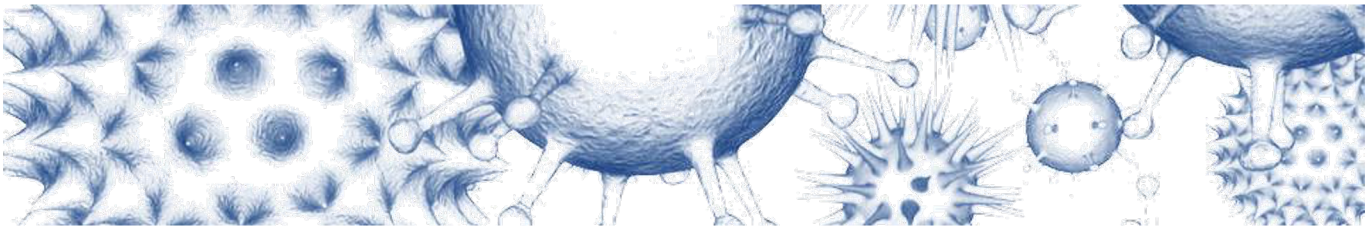


1 – IMMUNE RESPONSES TO SARS-CoV-2

Akiko Iwasaki

Department of Immunobiology, New Haven, CT, USA
Yale University School of Medicine, New Haven, CT, USA
Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA
Howard Hughes Medical Institute, Chevy Chase, MD, USA

The clinical presentation of COVID-19 involves a broad range of symptoms and disease trajectories. Understanding the nature of the immune response that leads to recovery over severe disease is key to developing effective treatments for COVID-19. In this talk, I will discuss immune responses in COVID-19 patients with moderate and severe disease. I will compare viral load, immune phenotype and cytokines that are predictive of mortality, and discuss signatures of cytokines and growth factors that associate with recovery vs. disease exacerbation. I will discuss impact of mutations of variants on vaccine-induced immunity, and the key adaptive immune players in clearance of primary infection and prevention of infection induced by vaccines. Finally, I will touch on long COVID disease pathogenesis and ongoing studies.

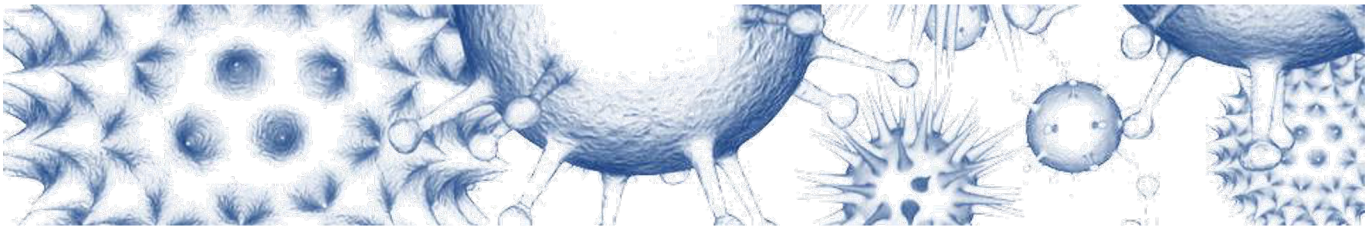


2 – ANTIVIRAL ACTIVITY OF SILYMARIN, BAICALEIN AND BAICALIN AGAINST DENGUE VIRUS

Low Zhao Xuan[1], Brian Ming OuYong[2], Pouya Hassandarvish[3], Chit Laa Poh[2], and Babu Ramanathan[1]*

[1]Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, Kuala Lumpur, Malaysia
[2]Centre for Virus and Vaccine Research, School of Medical and Life Sciences, Sunway University, Kuala Lumpur, Malaysia
[3]Tropical Infectious Diseases Research and Education Centre, University Malaya 50603, Kuala Lumpur, Malaysia

Dengue is an arthropod-borne viral disease that has become endemic and a global threat in many countries with no effective antiviral drug available currently. This study showed that flavonoids: silymarin and baicalein could inhibit the dengue virus *in vitro* and were well tolerated in Vero cells with a half-maximum cytotoxic concentration (CC_{50}) of 749.70 µg/mL and 271.03 µg/mL, respectively. Silymarin and baicalein exerted virucidal effects against DENV-3, with a selective index (SI) of 10.87 and 21.34, respectively. Baicalein but not silymarin could inhibit the DENV-3 progeny infectivity with a SI of 7.82. Baicalein effectively blocked DENV-3 attachment (95.59%) to the Vero cells, while silymarin prevented the viral entry (72.46%) into the cells, thus reducing viral infectivity. Both flavonoids showed promising antiviral activity against all four dengue serotypes. The *in silico* molecular docking showed that silymarin could bind to the viral envelope (E) protein with a binding affinity of -8.5 Kcal/mol and form hydrogen bonds with the amino acids GLN120, TRP229, ASN89, and THR223 of the E protein. Overall, this study showed that silymarin and baicalein exhibited potential anti-DENV activity and could serve as promising antiviral agents for further development against dengue infection.

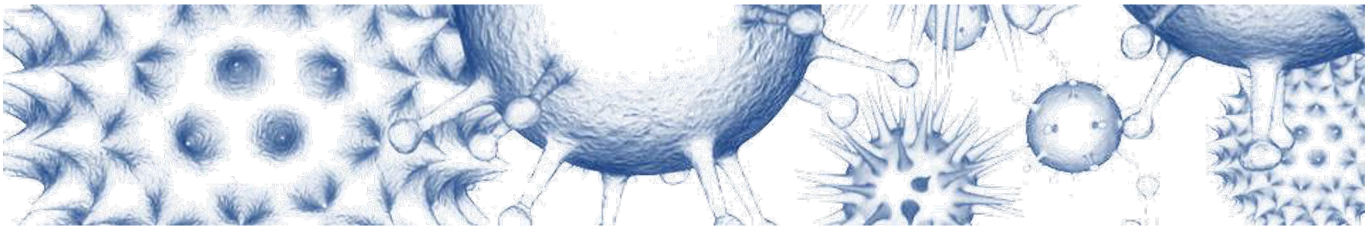


3 – IDENTIFICATION OF NOVEL NON-NUCLEOSIDE INHIBITORS OF DENGUE VIRUS NS5 RNA-DEPENDENT RNA POLYMERASE

Nicolò Santi[1], Silvia D'Andrea[1], Dirk Jochmans[2], Suzanne Kaptein[2], Salvatore Ferla[1], Johan Neyts [2]*, Andrea Brancale[1]*, and Giulio Nannetti[1]*

[1]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff CF10 3NB, Wales, U.K.
[2] KU Leuven-Laboratory of Virology and Chemotherapy, Department of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, Leuven 3000, Belgium

Dengue fever is a widespread febrile disease caused by any of the four serotypes of dengue virus (DENV), which can develop into life-threatening complications, known as severe Dengue. Currently, no antiviral treatment is available against DENV. An attractive target for the design of anti-DENV agents is represented by NS5 RNA-dependent RNA polymerase (RdRp), which performs several functions including *de novo* RNA synthesis, and it is highly conserved among different flaviviruses. Herein, we report the identification of novel anti-DENV agents that inhibit the enzymatic activity of DENV NS5 RdRp. Starting from the available DENV NS5 crystal structures, two distinct *in silico* screenings of 5 million drug-like compounds were carried out on the RdRp N-pocket to identify potential new non-nucleoside NS5 polymerase inhibitors. The ability of the selected compounds to interfere with the RdRp polymerase activity was then screened by a fluorescent-based *de novo* initiation (*dn*I) enzymatic assay. The active compounds showed to inhibit the polymerase activity at micromolar concentrations and in a dose-dependent manner. Moreover, the best compounds demonstrated the ability to inhibit the replication of DENV strains in antiviral cell-based assays at non-cytotoxic concentrations. Based on these findings, series of analogues of the best hit will be designed and synthesised to improve the potency of the antiviral activity. In summary, this study has enabled the identification of new DENV NS5 polymerase inhibitors endowed with novel molecular scaffolds that constitute a promising basis for the development of potential antiviral drug candidates for the treatment of DENV infections.

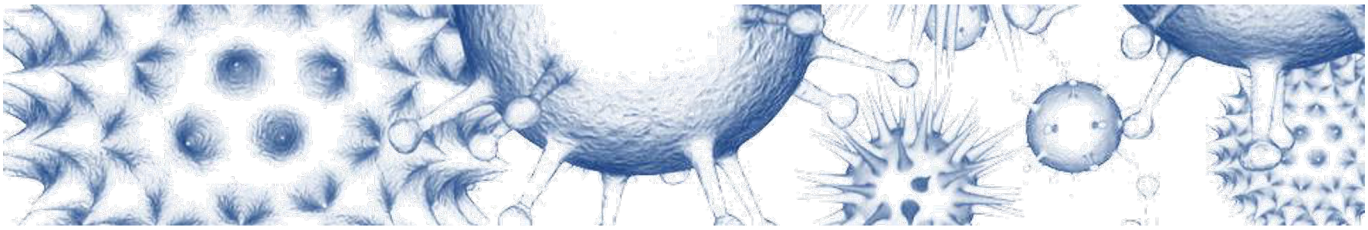


4 – AN APPLICATION OF THE OBTAINED COMPARISON RESULTS FROM THE ANALYSIS OF THREE CLASSES OF PATIENTS PHARMACOLOGICALLY TREATED AND UNTREATED TO THE STRUCTURE OF THE HIV-1 INTEGRASE TO OBTAIN A DEEPER UNDERSTANDING OF THE OBSERVED MUTATIONS

Saverio Lemme[1].

[1]*Phd Student of MIMIT Tor Vergata.*

The fight against AIDS is still an important problem that affects many people. The use of the combined antiretroviral therapy (cART) was an important step that allowed to transform HIV/AIDS into a manageable chronic condition, reducing the resulting morbidity and mortality. There remains the problem of drug resistance that develops during their use.
Regarding INSTIs, a study on integrase sequences was conducted starting from three classes of patients (drug-naïve, INI- naïve and INI experienced) in order to characterize and compare the observed mutations. Having available both the information on the observed mutations and on the structure of the integrase it is interesting to have a deeper understanding of what happens at a three-dimensional level as drug resistance using the methods of Structural Biology such as molecular docking and molecular dynamics.

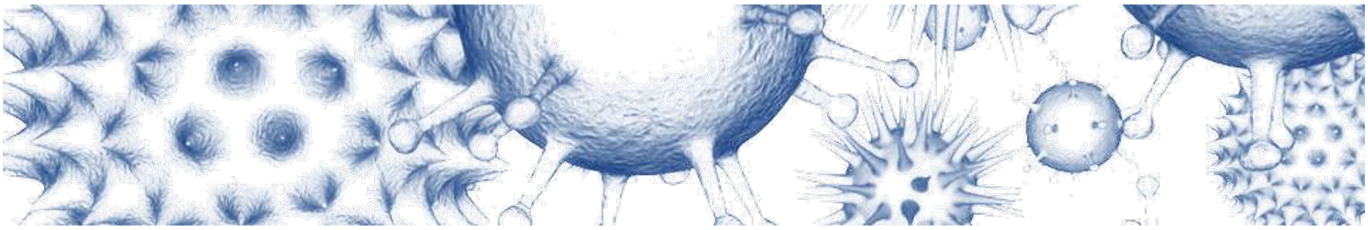


5 – DESIGN AND DEVELOPMENT OF NOVEL OXADIAZOLE BASED 1,3,5-TRIAZINES AS NNRTI TARGETING ENTRANCE CHANNEL OF NNRTI BINDING SITE

Singh Saumya[1], Bhat Hans Raj[2], Singh Udaya P.[1]

[1]Drug Design and Discovery Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad, Uttar Pradesh, India
[2]Department of Pharmaceutical Sciences, DIbrugarh University, Dibrugarh, Assam, India

HIV has affected the lives of millions of individuals around the globe. Despite need and an enormous amount of research, the cure has remained elusive due to the rapid onset of mutated forms. NNRTIs serve as the major backbone of currently used HAART therapy against HIV. However, the clinical significance of these NNRTIS is seriously compromised due to unfavourable aqueous solubility. Encouraged by the above, the present study attempts to develop novel aqueous soluble hybrid Oxadiazole based 1,3,5-triazines inspired by diaryltriazines (DATAs) to improve aqueous solubility. These molecules were developed using facile synthetic route in four-step reaction in excellent yield and purity. In docking analysis, compounds 4a, 4b, 4dd and 4aa were identified as the most potent inhibitors among the tested series. Docking results suggest that these inhibitors are found oriented in typical “butterfly” conformation in the active site of HIV-1 RT. The central triazine amine was found located at the entrance channel of the NNRTI binding pocket, a largely open solvent-exposed region in front of K101, E138, and V179 residues. The binding energies of compounds were found higher than that of reference ligand (Etravirine). Our study demonstrated the development of novel Oxadiazole based 1,3,5-triazines as novel NNRTI targeting the entrance channel of NNRTI binding pocket.



6 – CELL-BASED CIS-CLEAVAGE SCREENING METHOD FOR SARS-CoV-2 PROTEASE INHIBITORS

Heilmann E.[1*], Geley S.[2], Volland A.[1], Rupp B.[3], and von Laer D.[1*]

[1] Institute of Virology, Medical University of Innsbruck, Innsbruck, Austria
[2] Division of Pathophysiology, Medical University of Innsbruck, Innsbruck, Austria
[3] Institute of Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria

Virus protease inhibitors are among the most powerful antiviral drugs. However, for SARS-CoV-2 only few protease inhibitors have been identified so far and there is still a great need for assays that can efficiently screen for new SARS-CoV-2 protease inhibitors.
Here, we developed an imaging based photometric protease activity reporter assay in living cells based on a replication defective rhabdovirus. It evaluates the compounds cell permeability, toxicity and efficacy. Furthermore, the design of our assay is unique as it enables the measurement of cis-cleavage activity and by further modification even the distinction of N- and C-terminal cleavage. We thereby demonstrate that the SARS-CoV-1 and 2 protease inhibitor GC376 acts as an N- and C-terminal cis-cleavage inhibitor, whereas the HIV protease inhibitor boceprevir is a C-terminal cis-cleavage inhibitor. Our recent work with the HIV protease shows the flexibility of our platform and the possibility to extend it to virus proteases beyond those of SARS-CoV-2 and HIV.

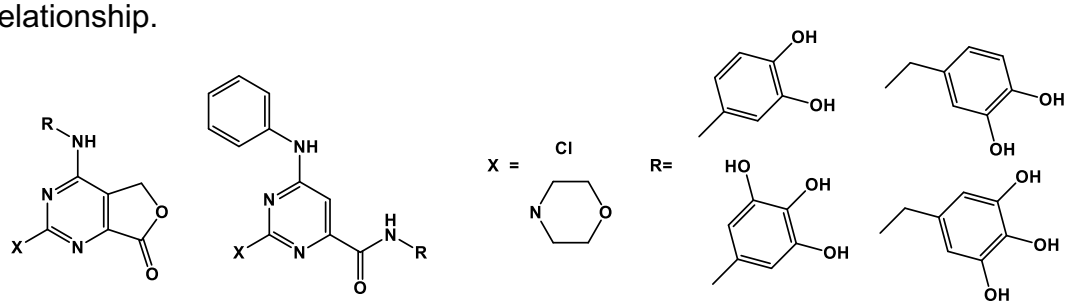
7 – SYNTHESIS OF POLYHYDROXYLATED HETEROCYCLIC COMPOUNDS AS INHIBITORS OF INFLUENZA VIRUS PAN ENDONUCLEASE

Francesca Miglioli[1], Mauro Carcelli[1], Dominga Rogolino[1], Marco Radi[2]

[1] Dipartimento di Scienze Chimiche, Della Vita e della Sostenibilità Ambientale Università degli Studi di Parma, Parco Area Delle Scienze 17/A, 43124, Parma, Italy
[2] Dipartimento di Scienze degli Alimenti e del Farmaco, Università degli Studi di Parma, Parco Area Delle Scienze 17/A, 43124, Parma, Italy

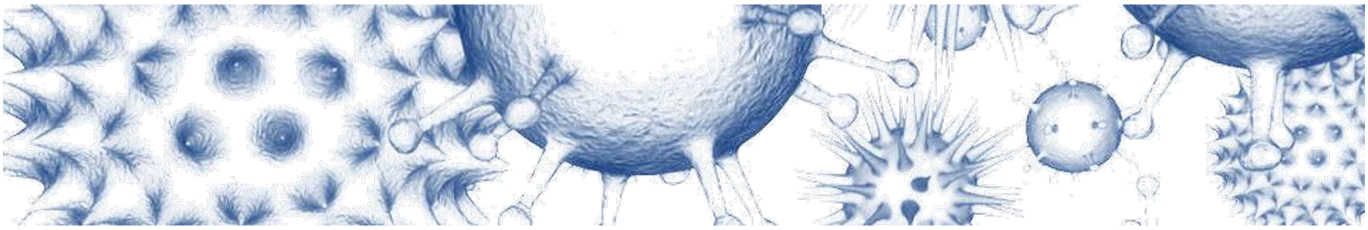
Influenza virus is cause of significant morbidity and mortality and the spread of influenza viruses resistant to conventional therapeutics enlighten the need for new drugs with different pharmacological targets. The influenza virus polymerase trimeric complex is an attractive target because of its fundamental role in the virus replication and its conserved structure among different strains. The N-terminal domain of PA (protein acidic) subunit is responsible for the endonuclease activity [1], and it contains one or more metal ions (Mg2+ or Mn2+) in the catalytic core of the enzyme. Thus, the metal ion chelation strategy has proved to be a useful tool for the discovery of potent PAN endonuclease inhibitors. Notably, Baloxavir Marboxil, prodrug of a chelating inhibitor of PAN endonuclease, was approved for the treatment of acute uncomplicated influenza [2]. Recently, starting from 2,4-dichlorofuropyrimidinone some furopyrimidinone and pyrimidine derivatives have been synthetized and showed inhibition towards dengue virus and zika virus replication, indicating that this intermediate can be useful in the synthesis of molecules with a biological relevance [3].

With this in mind, we synthetized a new set of molecules (**scheme 1**) with a furopyrimidinone or pyrimidine core substituted with aromatic di-and tri-hydroxylated amines, that can act as metal binding groups, exerting a possible inhibition effect on PAN. Enzymatic assays against influenza virus PAN endonuclease and other viral RNA-dependent RNA polymerases will be conducted to investigate their biological activity and their structure-activity relationship.



Scheme 1. Furopyrimidinone and pyrimidine polyhydroxylated derivatives.

[1] Yuan, P.; Bartlam, M.; Lou, Z.; Chen, S.; Zhou, J.; He, X.; Lv, Z.; Ge, R.; Li, X.; Deng, T.; Fodor, E.; Rao, Z.; Liu, Y. Crystal structure of an avian influenza polymerase PA(N) reveals an endonuclease active site. Nature, 2009, 458(7240), 909-913.
[2] Heo, Y-A. Baloxavir: first global approval. Drugs, 2018, 78(6), 693-697.
[3] Vincetti, P.; Kaptein, S. J. F.; Costantino, G.; Neyts, J.; Radi, M.; Scaffold Morphing Approach To Expand the Toolbox of BroadSpectrum Antivirals Blocking Dengue/Zika Replication. ACS Med. Chem. Lett. 2019, 10(4) 558–563

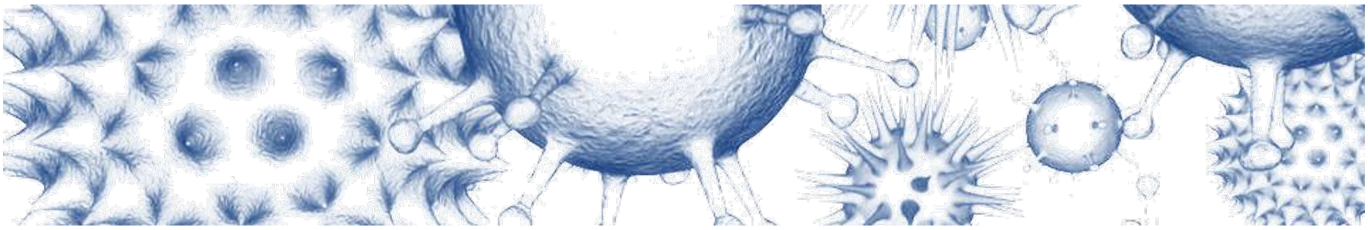


8 – LIPOPHILICALLY, BIOREVERSIBLY MASKED CYCLIC NUCLEOSIDE MONOPHOSPHATE DERIVATIVES - TOOLS FOR NON-INVASIVE CELL ASSAYS

Dominik Werz[1], Viachselav O Nikolaev[2], Andreas H Guse[2], Chris Gee[2], Chris Meier[1]

[1] Organic Chemistry, Department of Chemistry, Faculty of Science, University of Hamburg, Martin-Luther-King- Platz 6, D-20146 Hamburg.
[2] University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany

The second messenger cAMP is ubiquitously present in cells and is involved into various signalling processes. To transduce a signal, cAMP can either bind to distinctive cyclic nucleotide gated (CNG) ion channels or activate the protein kinases A. However, the role and the mechanism of action of different cNMPs in T cell regulation is not fully elucidated yet. A better understanding could enable the identification of novel targets for the treatment of autoimmune diseases. *In cell*-studies with cAMP or other cNMPs are difficult as their high polarity exclude them from passive membrane transport. Membrane-permeable cNMPs would enable such studies.
Starting from the nucleosides we synthesized different acyloxybenzyl-(AB)-masked cNMP derivatives on a short and flexible chemical route using phosphoramidite chemistry. Hydrolysis tests showed high chemical stability of the AB-cNMPs which was significantly higher than the rate of enzymatic activation. We evaluated the capability of AB-cNMPs to cross cell membranes and to deliver the AB-ester intracellularly. A FRET based cell assay in cardiomyocytes or hippocampal neurons proved the cellular uptake of AB-cAMP or AB-cGMP and the esterase catalysed activation. In a second set-up Jurkat T cells were loaded with a Ca2+ selective fluorescent dye and incubated in AB-cAMP derivatives. This assay confirmed the rapid uptake of AB-cAMPs into cells and the almost immediately occurring cellular response. Thus, we have developed novel membrane-permeable delivery systems for several highly polar, phosphorylated nucleoside second messengers for non-invasive studies on intracellular signalling.

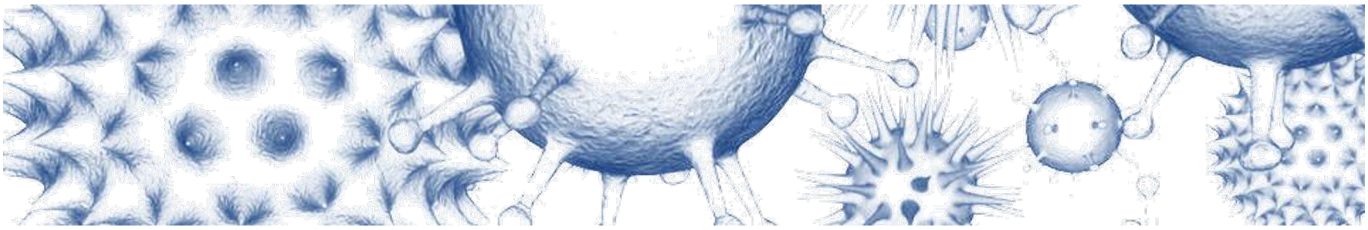


9 – THE DEVELOPMENT OF BROAD-SPECTRUM RECOMBINANT BETACORONAVIRUS VACCINE WITH PLANT VIRUS BASED PLATFORM ADJUVANT

Ekaterina M. Ryabchevskaya[1], Angelina O. Kovalenko, Tatiana I. Manukhova, Marina V. Arkhipenko, Ekaterina A. Evtushenko.

[1]Department of Virology, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russian Federation

Coronaviruses cause hard respiratory syndromes. SARS-CoV appeared in 2002, MERS-CoV – in 2012 and is still circulating in the Middle East, SARS-CoV-2 onset in 2019 led to the global pandemic. Considering the risk of the new highly pathogenic coronaviruses emergence, broad-spectrum coronavirus vaccine development is desirable. S-protein is the main coronaviruses' antigen and consists of two subunits. S1-subunit contains receptor-binding domain (RBD) being the main target of virus-neutralizing antibodies. S2-subunit includes two heptad repeats (HR1 and HR2) mediating membrane fusion. Here we designed the following recombinant antigens: (i) Co1 represented the consensus sequence of SARS-CoV-2 RBD-domain, (ii) PE – polyepitope protein consisted of highly conservative for SARS-like coronaviruses antigenic determinants of S2, (iii) M-protein represented the consensus sequence of MERS-CoV RBD-domain and (iv) CoF being the Co1 protein fused to the conservative S2 epitope located in HR2. Antigenic properties of these proteins were confirmed by western-blot analysis employing commercial (MyBioSourse, USA) polyclonal antibodies to the full-size S-protein of SARS-CoV (432054) and SARS-CoV-2 (434243) as well as to the S1-subunit of MERS-CoV (430258). As a platform-adjuvant, we used previously developed in our laboratory spherical particles (SPs), forming via tobacco mosaic virus heating. For SPs-antigens compositions contained simultaneously Co1, PE and CoF, the clear recognition by both above-mentioned SARS-specific antibodies was demonstrated by immunofluorescence analysis. Their immunogenicity was analyzed in a mice model; SPs were proved to increase antibody titers to the coronaviruses' antigens. Therefore, obtained recombinant antigens and their compositions with SPs are a promising base for broad-spectrum coronavirus vaccine. Funding: RFBR №20-04-60006.

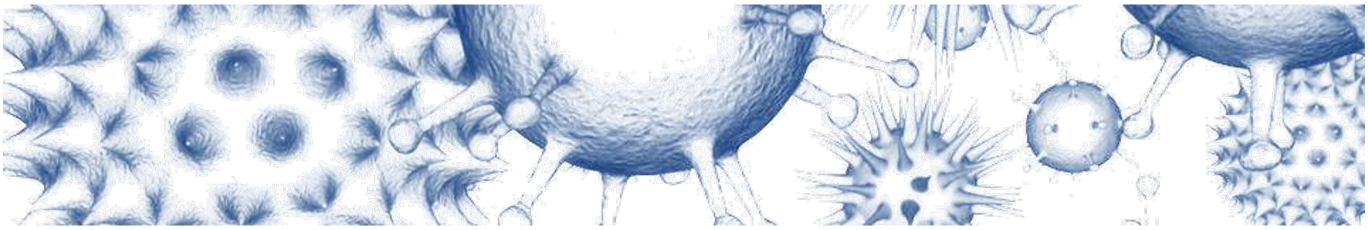


10 – FLAVONOIDS INDUCE TYPE I INTERFERON EXPRESSION THROUGH STING DEPENDENT PATHWAY

A. Paulis[1], P.O. Vidalain[2], V. Lotteau[2], S. Distinto[1], F. Cottiglia[1], E. Maccioni[1] and E. Tramontano[1]

[1]Department of Life and Environmental Sciences, University of Cagliari, 09042 Monserrato, Italy
[1]CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, F-69007, France

The hSTING is a transmembrane protein localized in the endoplasmic reticulum (ER) involved in the innate immune response by stimulating type I Interferons (INFs) transcription. When cytosolic DNA is detected, cGAS (cyclic GMP-AMP synthase) produces 2'3' cGAMP (2'3' cyclic GMP-AMP), such cyclic dinucleotide binds hSTING triggering IFN Regulatory Factor 3 (IRF3) phosphorylation and dimerization, this complex is translocated into the nucleus where stimulate type I IFN (IFN-I) transcription. Moreover, hSTING recognizes bacterial cyclic dinucleotide 3'3' cGAMP, demonstrating its relevance during both viral and bacterial infections. hSTING has a role also against tumorigenesis. Recent studies identified hSTING agonists which showed antiviral and antitumor activity, for this reason, a selection of putative STING-ligands is tested to evaluate their ability to induce IFNs. Starting from a small in house library through a virtual screening approach we identified four flavonoids potentially interacting with STING. A gene reporter assay with cells knock-out for STING (HEK293T) has been used to investigate compounds' dependent IFN-β transcription in presence of reconstituted hSTING and a luciferase reporter gene driven by the human IFN-β promoter. We determined the ability the tested compounds to induce IFN-β transcription. Next, they were tested in two gene-reporter systems based on HEK293T and Huh7 cells, stably transfected with a luciferase gene driven by the ISRE promoter, that express endogenous STING in presence and absence of IFN α. Two of the four identified flavonoids strongly boosted IFN-I signalling. Since it is known that pyrimidine biosynthesis is strongly active during cancer and viral infections, making the dihydroorotate dehydrogenase, the key enzyme in pyrimidine biosynthesis, a target of antivirals and anticancer drugs, we performed an assay to evaluate the ability of the compounds to block pyrimidine biosynthesis. The identification of selective STING ligands with agonist activity, could constitute a starting point in the development of molecules as immunotherapeutic against viral infections and cancer.



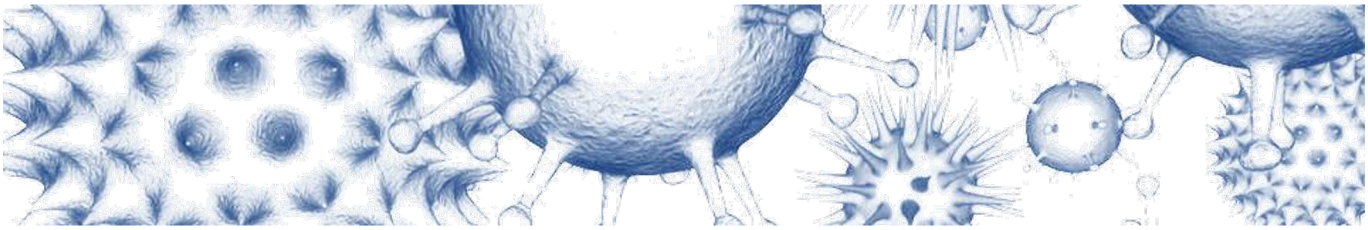
11 – EXPLORING THE IMPLICATION OF DDX3X IN DENV INFECTION: DISCOVERY OF THE FIRST-IN-CLASS DDX3X FLUORESCENT INHIBITOR

Annalaura Brai[1], Adele Boccuto,[2] Martina Monti[3], Serena Marchi[3], Francesco Saladini[2], Claudia Immacolata Trivisani[1], Claudia Maria Trombetta[3], Emanuele Montomoli[3], Valentina Riva[5], Maurizio Zazzi[2], Giovanni Maga[5], Elena Dreassi[1] and Maurizio Botta[1]

[1] Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Italy.
[2] Dipartimento di Biotecnologie Mediche, Università degli Studi di Siena, Italy
[3] Dipartimento di Medicina Molecolare e dello Sviluppo, Università degli Studi di Siena, Italy
[5] Institute of Molecular Genetics IGM-CNR "Luigi Luca Cavalli-Sforza", Italy

In the absence of effective drugs or vaccines for the treatment of the five Dengue virus (DENV) serotypes, the search of novel antiviral drugs is of primary importance for the scientific community. In this context, the study of host targets is now attracting attention since it allows to identify broad-spectrum drugs endowed with a high genetic barrier. In the last ten years, our research group identified several small molecules DDX3X inhibitors[1-3] and proved their efficacy against different viruses including novel emerging ones. Herein, we focused our efforts on expanding the structure-activity relationship (SAR) around the two series of DDX3X inhibitors, concentrating our work on the search of novel promising compounds active against DENV infection. As a result, we discovered novel DDX3X helicase inhibitors with improved antiviral activity, comparable or lower than those reported for known broad-spectrum antivirals such as ribavirin or sofosbuvir. Notably, the most promising derivative is about 9-times more active than the previous hit. In addition, we investigated the mechanism of action of our compounds in infected cells, synthesizing a novel fluorescent derivative namely 25. Immunofluorescence analysis confirms that 25, during the first hours of DENV infection, co-localized with DDX3X promoting the reduction of NS5 positive cells and recovering the cell number, over the time. The low cytotoxicity of compounds, indicates once again that our compounds are characterized by high cellular tolerability. Overall, our results confirm that DDX3X inhibitors represent a safe and promising class of antivirals, supporting their evaluation in an animal model of DENV infection.

[1] Brai, A.; Fazi, R.; Tintori, C.; Zamperini, C.; Bugli, F.; Sanguinetti, M.; Stigliano, E.; Esté, J.; Badia, R.; Franco, S.; et al. Human DDX3 Protein Is a Valuable Target to Develop Broad Spectrum Antiviral Agents. Proc. Natl. Acad. Sci. U. S. A. 2016, 113 (19), 5388–5393.
[2] Brai, A.; Riva, V.; Saladini, F.; Zamperini, C.; Trivisani, C. I.; Garbelli, A.; Pennisi, C.; Giannini, A.; Boccuto, A.; Bugli, F.; et al. DDX3X Inhibitors, an Effective Way to Overcome HIV-1 Resistance Targeting Host Proteins. Eur. J. Med. Chem. ***2020****, 200.*
[3] Brai, A.; Ronzini, S.; Riva, V.; Botta, L.; Zamperini, C.; Borgini, M.; Trivisani, C. I.; Garbelli, A.; Pennisi, C.; Boccuto, A.; et al. Synthesis and Antiviral Activity of Novel 1,3,4-Thiadiazole Inhibitors of DDX3X. Mol. 2019, Vol. 24, Page 3988 2019, 24 (21), 3988.

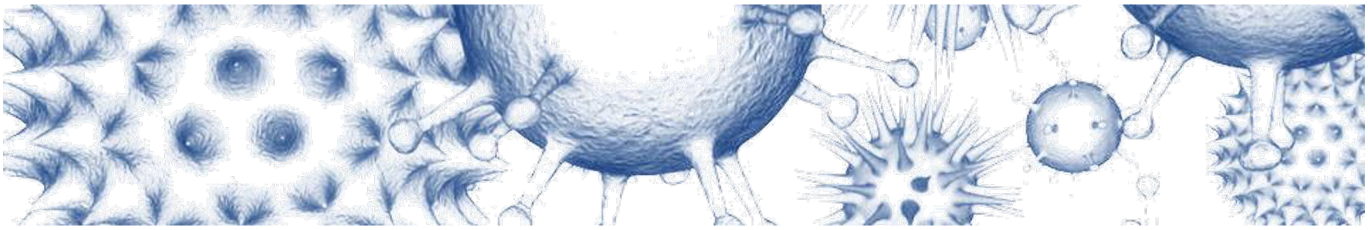


12 – AN AMYLOIDOGENIC FRAGMENT OF HUMAN ALPHA HEMOGLOBIN WITH A COMBINED ANTIBACTERIAL AND ANTIVIRAL ACTIVITY

Lia-Raluca Olari[1], Richard Bauer[2], Laura Cortez-Rayas[3], Rüdiger Groß[1], Carina Conzelmann[1], Armando A. Rodríguez Alfonso[4], Fabian Zech[1], Lena Steppe[5], Nico Preising[4], Ludger Ständker[4], Jens von Einem[3], Barbara Spellerberg[2], Jan Münch[1,4*]

Institute of Molecular Virology, University Clinic of Ulm, 89081 Ulm, Germany
[2] Institute of Medical Microbiology and Hygiene, University Clinic of Ulm, 89081 Ulm, Germany
[3]Institute of Virology, University Clinic of Ulm, 89081 Ulm, Germany
[4]Core Facility for Functional Peptidomics, Ulm Peptide Pharmaceuticals (U-PEP), University Clinic of Ulm, 89081 Ulm
[5]Institute of Orthopaedic Research and Biomechanics, University Clinic of Ulm, 89081 Ulm, Germany

Antimicrobial peptides (AMPs) are essential components of the immune system, defending against invasive microbial infections. AMPs use different strategies, including the self-assembly into amyloid-like structures. To identify peptides protecting from microbial infections, we screen peptide libraries from human body fluids and organs for amyloid formation and antiviral activity. Testing a human spleen library, allowed to identify an amyloidogenic peptide, later revealed by mass spectrometry as the C-terminal region of human alpha hemoglobin, termed HBA (111-142). The peptide forms positively charged fibrils by agitation at 37°C. The freshly dissolved peptide acts potently against gram negative (*P. aeruginosa*, *A. baumanii*) and gram positive (*Listeria monocytogenes, Enterococcus faecium*) bacteria. In contrast, HBA (111-142) fibrils, but not the freshly dissolved peptide, inhibited Herpes Simplex-2 (HSV-2) infection in a dose-dependent manner, with a mean IC50 of ~100 µg/ml. Interestingly, the fibrils seem to have a specificity against Herpesviruses, inhibiting HSV-1, HSV-2, and Human cytomegalovirus (HCMV), but having no effect on ZIKV or SARS-CoV-2. Hemoglobin is the second most abundant human protein, well known for generating peptides, especially under acidic conditions, a hallmark of infection and inflammation. Indeed, we found that HBA (111-142) is released by Pepsin and Napsin A, acidic proteases expressed in spleen and other organs. Even if only ~5% of available hemoglobin is digested, values would correspond to ~900µg/ml, sufficient to block HSV infection or bacterial growth entirely.

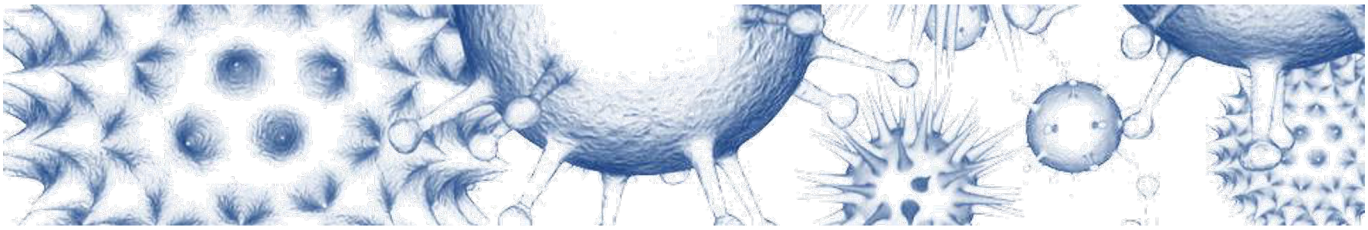


13 – NOVEL ANTIVIRAL ACTIVITY OF PADs INHIBITORS AGAINST BETA-CORONAVIRUSES SARS-CoV-2 AND HCoV-OC43

Selina Pasquero [1], Gloria Griffante [3], Francesca Gugliesi [1], Matteo Biolatti [1], Camilla Albano [1], Sergio Fernando Castillo Pacheco [1], Greta Bajetto [1], Valentina Dell'Oste [1], Serena Del Bue [4], Silvia Parapini [4], Santo Landolfo [1], Marco De Andrea [1,2]

[1] Department of Public Health and Pediatric Sciences, University of Turin, 10126 Turin, Italy
[2] CAAD Center for Translational Research on Autoimmune and Allergic Disease, Novara Medical School, 28100 Novara, Italy.
[3] Department of Translational Medicine, University of Eastern Piedmont, 28100Novara, Italy
[4] Department of Biomedical, Surgical and Dental Sciences, University of Milan, 20122 Milan, Italy

Novel coronaviruses (CoVs) have repeatedly attracted the attention of researchers over the past few decades. To date, seven human CoVs (HCoVs) have been identified: among them, HCoV-OC43 and SARS-CoV-2, the causative agent of the ongoing epidemic of atypical pneumonia (COVID-19), belong to beta genus. A recent study described the putative roles of a family of enzymes called peptidylarginine deiminases (PADs) in COVID-19 disease. PADs are a family of cellular enzymes that catalyze the post-translational modification citrullination, a process in which the guanidinium group of a peptidyl-arginine is hydrolyzed to form peptidyl-citrulline, a non-genetically coded aminoacid. PADs dysregulation leads to an aberrant citrullination which is a characteristic biomarker of several inflammatory conditions. Based on these evidences, the aim of this work was to evaluate whether PAD inhibitors were a reliable new class of host-targeted antivirals against coronaviruses.
By using the HCoV-OC43 and SARS-CoV2 strains as models of infection in human lung fibroblasts (MRC-5) and monkey kidney cells (Vero-E6), we observed that the pharmacological inhibition of PAD enzymes led to a significant reduction of viral replication. Furthermore, the overall citrullination profile obtained with the citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe changes consistently during infection. Interestingly, this is associated with an increase of PAD expression, both at mRNA and protein levels. Taken together our results suggested that i) citrullination is a process that can be induced by beta-coronaviruses, as a mechanism to foster their replication, and 2) that increase of PADs activity is central for beta-coronavirus replication.

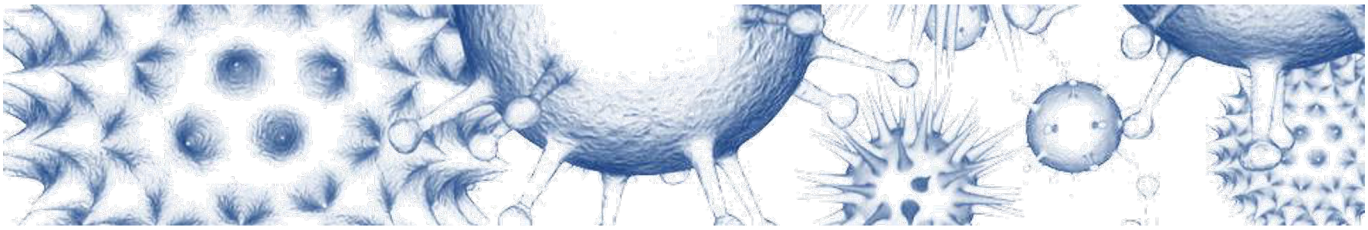


14 – HERPESVIRUS DNA POLYMERASES AND IMPACT OF MUTATOR PHENOTYPES

Graciela Andrei

Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Herestraat 49 bus 1043, 3000 Leuven, Belgium

Herpesviruses are large DNA viruses that replicate their genomes using their own DNA polymerases (DNA pols). Herpesvirus DNA pols belong to the B-family of DNA pols (also known as α-like DNA pols), which share seven regions of homology denoted I to VII and a δ-region C (homologous to DNA pol δ). Because herpesvirus DNA pols are equipped with a proofreading 3'-5' exonuclease, they have lower mutation rates and are thought to be genetically more stable than RNA viruses. However, recent data suggest that both diversity and evolution of herpesviruses may be impacted by several mechanisms, conferring them increased variability. Some mutations in α-like DNA pols can affect DNA replication fidelity. For instance, specific substitutions in the 3'→5' exonuclease decrease the proofreading capacity, causing higher mutation rates, a so-called mutator phenotype. Contrarily, other mutations in this region increase exonuclease activity improving replication fidelity, known as antimutator phenotype. Specific changes in the 3'→5' exonuclease region of human DNA pols are known mutator phenotypes that contribute to carcinogenesis by inducing a large number of mutations and genomic instability. Among DNA viruses, a mutator phenotype has been described in bacteriophage T4, in poxviruses and more recently in some human herpesviruses (HHVs). We recently identified two novel amino acid changes [C297W (3'-5' exonuclease domain) and C981Y (thumb domain)] in murine gammaherpesvirus 68 (MHV-68) DNA pol, inducing increased spontaneous mutations. The association of the C297W with a mutator phenotype was validated by CRISPR/Cas9 genome editing. Competitive fitness of C297W and C981Y mutant viruses with/without antivirals was significantly impaired as evaluated following population evolution by next-generation sequencing. The emergence of a mutator phenotype in HHVs in immunocompromised patients may have important consequences for antiviral therapy efficacy and overall outcome of the patients. Through our translational platform RegaVir, we identified several substitutions of (un)known significance in the HHV DNA pol 3'-5'-exonuclease domain potentially linked to a mutator phenotype that should be further investigated for their potential association with an increased mutation rate as homologous positions are associated with a known mutator phenotype in human and/or S. cerevisiae DNA pols δ and/or ε.

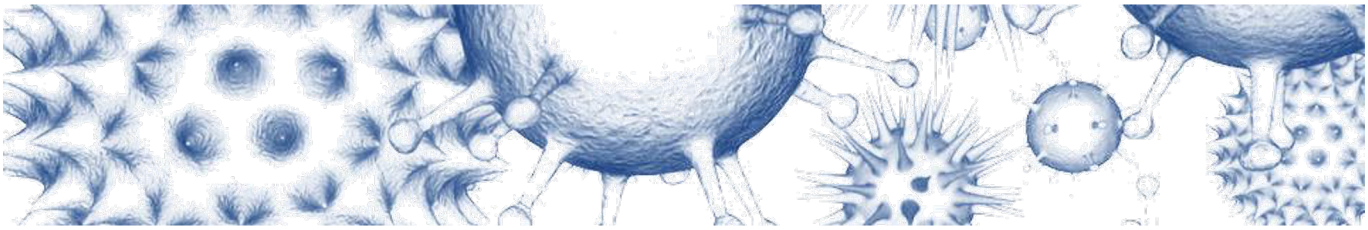


15 – CONTROLLED ADMINISTRATION OF AEROSOLIZED SARS-CoV-2 TO K18-hACE2 TRANSGENIC MICE UNCOUPLES RESPIRATORY INFECTION AND ANOSMIA FROM FATAL NEUROINVASION

Matteo Iannacone

Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy.
Vita-Salute San Raffaele University, Milan, Italy.
Experimental Imaging Centre, IRCCS San Raffaele Scientific Institute, Milan, Italy.

The development of a tractable small animal model faithfully reproducing human COVID-19 pathogenesis would arguably meet a pressing need in biomedical research. Thus far, most investigators have used transgenic mice expressing the human ACE2 in epithelial cells (K18-hACE2 transgenic mice) that are intranasally instilled with a liquid SARS-CoV-2 suspension under deep anesthesia. Unfortunately, this experimental approach results in disproportionate high CNS infection leading to fatal encephalitis, which is rarely observed in humans and severely limits this model's usefulness. Here, we describe the use of an inhalation tower system that allows exposure of unanesthetized mice to aerosolized virus under controlled conditions. Aerosol exposure of K18-hACE2 transgenic mice to SARS-CoV-2 resulted in robust viral replication in the respiratory tract, anosmia, and airway obstruction, but did not lead to fatal viral neuroinvasion. When compared to intranasal inoculation, aerosol infection resulted in a more pronounced lung pathology including increased immune infiltration, fibrin deposition and a transcriptional signature comparable to that observed in SARS-CoV-2-infected patients. This model may prove useful for studies of viral transmission, disease pathogenesis (including long-term consequences of SARS-CoV-2 infection) and therapeutic interventions.

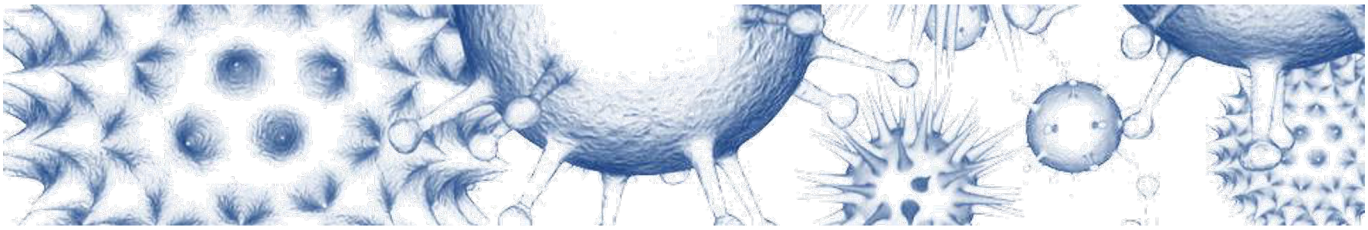


16 – USING RECOMBINANT HUMAN ANGIOTENSIN-CONVERTING ENZYME 2 (RHACE-2) AS ANTIVIRAL AGAINST SARS-COV 2”

Ali Mirazimi

Department of Microbiology, Public Health Agency of Sweden, SE-171 82, Solna, Sweden
Department of Laboratory Medicine, Karolinska University Hospital and KI, SE-14186, Huddinge, Stockholm, Sweden.
National Veterinary Institute, SE-756 51, Uppsala, Sweden.

It has been previously provided the first genetic evidence that Angiotensin converting enzyme 2 (ACE2) is the critical receptor for SARS-CoV and that ACE2 protects from lung in jury, providing a molecular explanation for the severe lung failure and death due to SARS-CoV infections. ACE2 has now also been identified as a key receptor for SARS-CoV-2 infections and it has been proposed that inhibiting this interaction might be used to treat patients with COVID-19. Here we show that clinical grade hrsACE2 reduced SARS-CoV-2 recovery from Vero cells by a factor of 1,000-5,000. An equivalent mouse rsACE2 had no effect, confirming previous studies. We also show that SARS-CoV-2 can directly infect engineered human kidney and intestinal organoids. These data show the virus can infect and replicate in human kidneys, and intestines (with shedding of the virus in the urinary tract and intestine) potentially leading to multi-organ failure. Most importantly we demonstrate that hrsACE2 can significantly block SARS-CoV-2 infections.



17 – DESIGN OF SELECTIVE HIV-1 RNASE H INHIBITORS STARTING FORM DKA DUAL INHIBITORS OF IN AND RNASE H

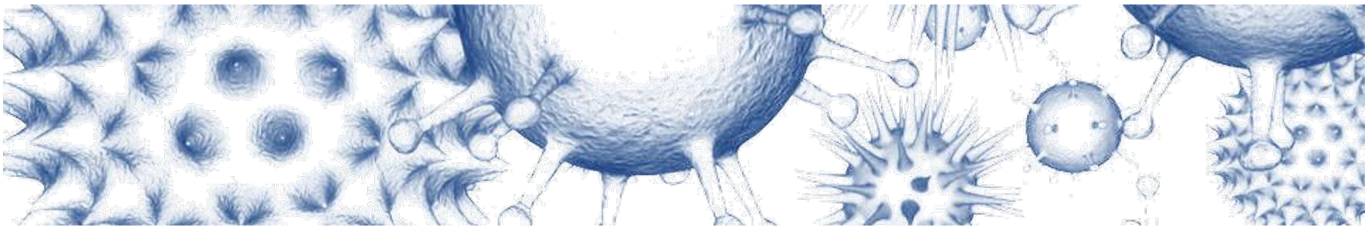
Roberto Di Santo

Dipartimento di Chimica e Tecnologie del Farmaco, Istituto Pasteur-Fondazione Cenci Bolognetti, "Sapienza" Università di Roma, p.le Aldo Moro 5, I-00185, Rome
roberto.disanto@uniroma1.it

The conversion of the viral RNA into DNA is a critical step in the HIV-1 life cycle initiated by the reverse transcriptase (RT), a well-established drug target. RT is a multifunctional enzyme with RNA and DNA-dependent DNA polymerase (RDDP and DDDP respectively), and ribonuclease H (RNase H) activities. RNase H function is essential for virus replication since it specifically cleaves the RNA moiety of RNA/DNA hybrid to generate a DNA duplex to be integrated into the host cell. The RNase H active site contains a highly conserved DEDD motif consisting of four carboxylate amino acid residues in close proximity (D443, E478, D498, and D549) that interact with two Mg^{2+} ions[1].

Despite being a valid and promising drug target, RNase H inhibitors have not reached the clinical pipeline yet. Indeed, all the RT-targeting drugs approved so far are inhibitors of the RDDP activity and the development of RNase H inhibitors (RHIs) has lagged behind so that no drug targeting RNase H has been approved yet. This can be attributed to: i) the availability of expertise on inhibitors of other DNA polymerases that encouraged the development of drugs targeting the RT- RDDP function, and ii) the open morphology of the RNase H function hard to target, and showing a strong competition with the substrate for the access to the catalytic core[1]. Recently we described a dual activity of DKA derivatives as inhibitors of both integrase and RNase H function of RT[2,3]. Herein we describe the application of an isosteric approach to convert these metabolically unstable DKA to more drug-like scaffolds[4]. This approach has been successful and also led to inhibitors that selectively target RNase H function vs IN enzyme.

[1] V. N. Madia, A. Messore, A. De Leo, V. Tudino, I. Pindinello, F. Saccoliti, D. De Vita, L. Scipione, R. Costi, R.Di Santo, Curr. Med. Chem. 2021, 28, 1.
[2] R. Costi, M. Metifiot, F. Esposito, G. Cuzzucoli Crucitti, L. Pescatori, A. Messore, L. Scipione, S. Tortorella, L. Zinzula, E. Novellino, Y. Pommier, E. Tramontano, C. Marchand, R. Di Santo, J. Med. Chem. 2013, 56, 8588.
[3] L. Pescatori, M. Métifiot, S. Chung, T. Masoaka, G. Cuzzucoli Crucitti, A. Messore, G. Pupo, V. N. Madia, F. Saccoliti, L. Scipione, S. Tortorella, F. Di Leva, F. S. Cosconati, L. Marinelli, E. Novellino, S. F. Le Grice, Y. Pommier, C. Marchand, R. Costi, R. Di Santo, J. Med. Chem. 2015, 58, 4610.
[4] A. Messore, A. Corona, V.N. Madia, F. Saccoliti, V. Tudino, A. De Leo, L. Scipione, D. De Vita, G. Amendola, S. Di Maro, E. Novellino, S. Cosconati, M. Métifiot, M. L. Andreola, P. Valenti, F. Esposito, N. Grandi, E. Tramontano, R. Costi, R. Di Santo, ACS Med. Chem. Lett. 2020, 11, 798.

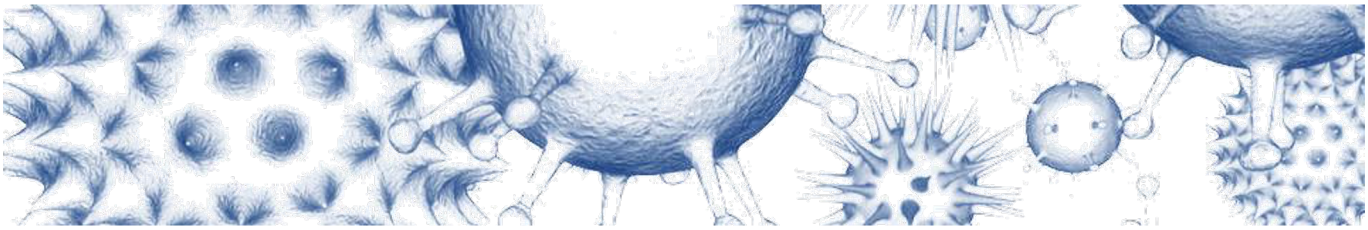


18 – STRUCURE-BASED DRUG DESIGN IN ANTIVIRAL DRUG DISCOVERY

Andrea Brancale

School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK

Drug discovery is a long, expensive, and often very frustrating process, for the researchers involved. For this reason, scientists are always looking for new approaches to improve this activity, making it less risky and less time consuming. At the very early stages of hit discovery and hit-to-lead, one of of the strategies that is often used is computer-aided drug-design (CADD). This includes a very fast, and very economical, set of techniques that allow the evaluation novel potential drugs in silico before committing to their synthesis in the lab. In this presentation, we will look at some of CADD applications (in particular, structure-based drug design) that have been used in designing new antivirals, discussing the advantages and disadvantages of such techniques.

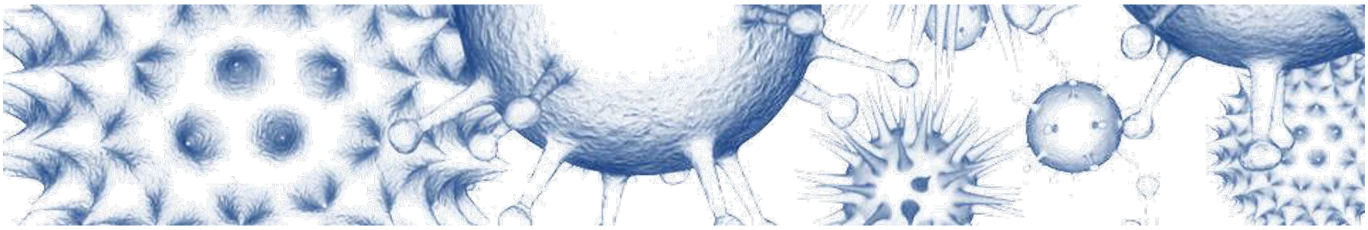


19 – STRUCTURAL APPROACHES TO SARS-COV-2 THERAPY AND STRATEGIES TO ADDRESS THE INEVITABILITY OF RESISTANCE

Jason McLellan

Department of Molecular Biosciences, University of Texas, Austin, TX 78712, USA

During this presentation I will discuss my laboratory's collaborative work understanding the structure and function of coronavirus spike proteins, and how we leveraged this information to design novel vaccine antigens that are in four leading COVID-19 vaccines. I will also describe how we were able to rapidly determine the structure of the SARS-CoV-2 spike protein in early 2020 and leverage that information into the design of second-generation spikes that are more stable and express better than our initial antigen. I will also discuss our recent work on spike-directed antibodies and strategies to address the inevitability of resistance.

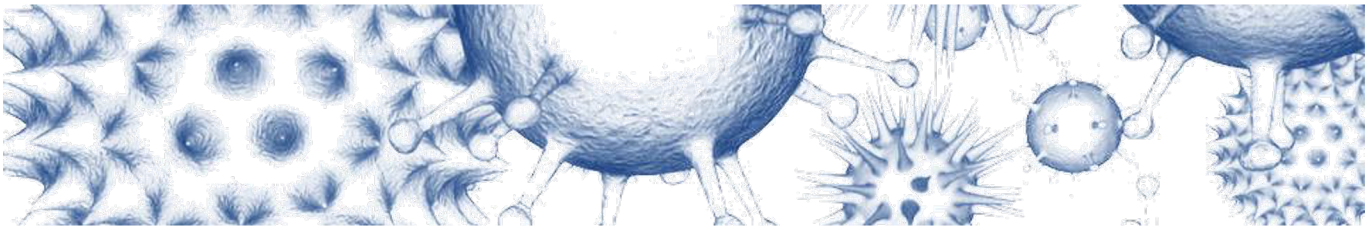


20 – INNOVATIVE HERPESVIRAL MECHANISMS TO ESCAPE RESTRICTION BY CELLULAR APOBEC ENZYMES

Reuben S. Harris

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota, USA, 55455
Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA, 55455
Institute for Molecular Virology, University of Minnesota, Minneapolis, Minnesota, USA, 55455
Howard Hughes Medical Institute, University of Minnesota, Minneapolis, Minnesota, USA, 55455

The APOBEC family of DNA cytosine deaminases provides a broad and overlapping defense against viral infections. Successful viral pathogens, by definition, have evolved strategies to escape restriction by the APOBEC enzymes of their hosts. HIV-1 and related retroviruses are thought to be the predominant natural substrates of APOBEC enzymes due to obligate single-stranded DNA replication intermediates, abundant evidence for cDNA strand C-to-U editing (genomic strand G-to-A hypermutation), and a potent APOBEC degradation mechanism. In contrast, much lower mutation rates are observed in double-stranded DNA herpesviruses and the evidence for APOBEC mutation has been less compelling. However, our recent work has revealed that Epstein-Barr virus (EBV), Kaposi's sarcoma herpesvirus (KSHV), and herpes simplex virus-1 (HSV-1) are potential substrates for cellular APOBEC enzymes. To prevent APOBEC-mediated restriction these viruses have repurposed their ribonucleotide reductase (RNR) large subunits to directly bind, inhibit, and relocalize at least two distinct APOBEC enzymes – APOBEC3B and APOBEC3A. The importance of this interaction is evidenced by genetic inactivation of the EBV RNR (BORF2), which results in lower viral infectivity and higher levels of C/G-to-T/A hypermutation. This RNR-mediated mechanism therefore likely functions to protect lytic phase viral DNA replication intermediates from APOBEC-catalyzed DNA C-to-U deamination. The RNR-APOBEC interaction defines a new host-pathogen conflict that the virus must win in real-time for transmission and pathogenesis. However, partial losses over evolutionary time may also benefit the virus by providing mutational fuel for adaptation.

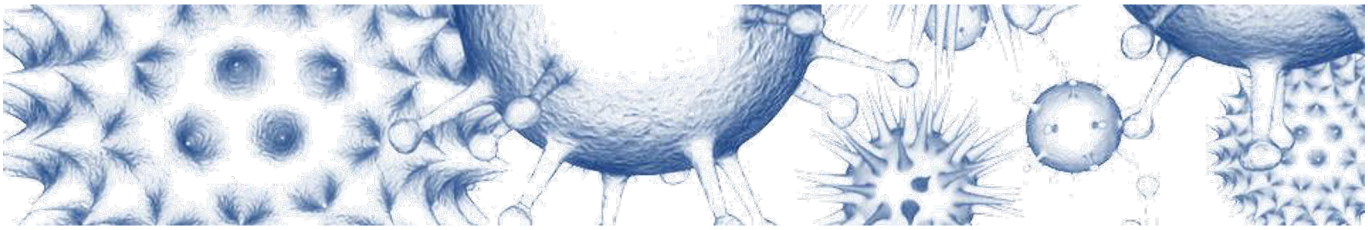


21 – RATIONAL (AND SOMETIMES IRRATIONAL!) STRATEGIES IN NUCLEOSIDE/NUCLEOTIDE DRUG DESIGN

Katherine Seley-Radtke

University of Maryland, Baltimore County (UMBC), Department of Chemistry & Biochemistry, Baltimore, MD 21250

Nucleosides and nucleotide analogues have a long and rich history in the field of medicinal chemistry, and they continue to play an essential role in the treatment of diseases. As a result, the pursuit of novel nucleoside analogues is critical to solving global health issues such as emerging and reemerging infectious diseases and other pathogens. The naturally occurring nucleosides represent a unique starting point for drug design due to their involvement in numerous critical biological processes as well as the fact that they serve as essential building blocks for both DNA and RNA synthesis. Because of this, modifications to their structure can be designed in and/or refined, based on ~~the~~ key interactions identified in the binding site of target enzymes. As the field has progressed and new information has become available about nucleoside structure, enzyme recognition, and biological activity, new and more complex modifications have been pursued, including multiple modifications on the same scaffold. As a result of these modifications, there are currently more than 30 nucleoside/tide analogues on the market approved for use in treating viruses, cancers, parasites, as well as bacterial and fungal infections, with many more in clinical and preclinical trials. This overview will provide a flavor of the historical progression of nucleoside development and the various types of unique structural modifications that have been pursued to date including the novel fleximer scaffold developed in the Seley-Radtke laboratories.

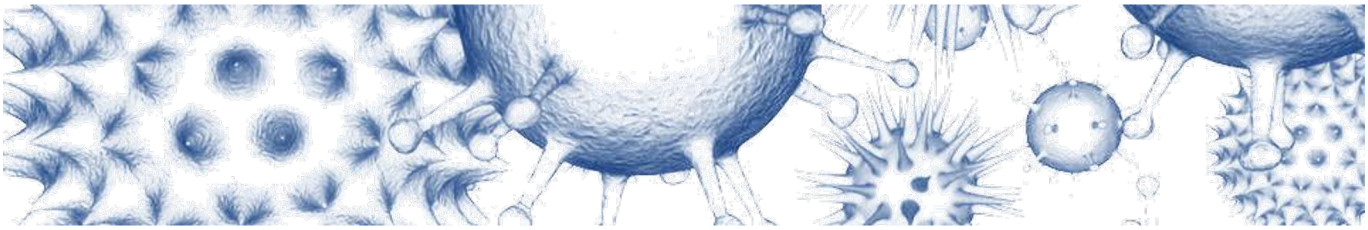


22 – ELUCIDATING THE PATHOGENIC MECHANISMS OF CHIKUNGUNYA VIRUS

St. Patrick Reid

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198-5900, USA

Chikungunya virus (CHIKV) is a re-emerging mosquito-borne alphavirus in the Togaviridae family of viruses. CHIKV is the etiologic agent of chikungunya fever (CHIKF), a disease characterized by high fever and debilitating polyarthralgia and polyarthritis that can typically last for 1-4 week. In some cases, individuals develop chronic disease that can last for months to years following infection. Long term chronic CHIKF is associated with both joint and bone manifestation of disease. Currently there are no approved vaccines or therapeutics available to prevent or treat CHIKV at the acute or chronic stages of disease. Greater understanding of the mechanism underlying the pathophysiology of the CHIKV infection will be useful in the development of effective therapeutics. Owing to the current deficiencies in knowledge, we propose a 3D human cell based-bone model to better understand the cellular mechanism of CHIKV disease and potential therapeutic targets.



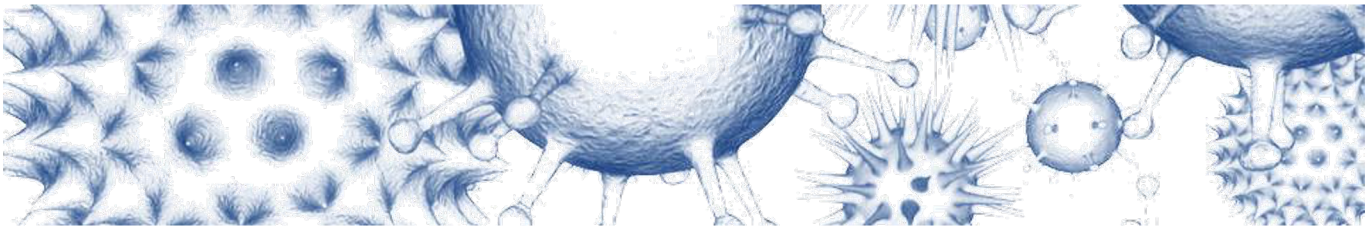
23 – STRUCTURAL & FUNCTIONAL INVESTIGATIONS OF A MURDEROUS VIRAL ENZYME IN A NATIVE-LIKE CONTEXT

Peter G. Stockley

Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, LS2 9JT, UK.

Abstract: Since its discovery, The World Health Organisation estimates that over 2 billion people have been infected by Hepatitis B virus (HBV), with >350 million becoming chronic carriers, leading to ~1 million HBV-related deaths annually. Treatments for cirrhosis and liver cancer are life-long and expensive, and ultimately they fail to prevent progression to liver cancer.

We showed previously that HBV, a para-retrovirus that initially assembles its nucleocapsid (NCP) around a ssRNA version of its genome, does so using a mechanism we have characterised in *bona fide* ssRNA viruses. Multiple, short, dispersed sequences/motifs across these genomes (gRNAs) each have affinity for their cognate coat proteins (CPs). These RNA Packaging Signals (PSs) then act collectively to ensure faithful assembly in complex cellular milieus, and provide genetic resilience against mutation. The HBV PSs may also play a role in ensuring the virally-encoded polymerase (Pol) has a non-entangled substrate to work on, as it converts the ss pre-genomic RNA to ssDNA, whilst degrading the template and then subsequently creating a dsDNA version, all within the confines of the NCP. Small drug-like ligands binding to HBV PSs ablate in vitro NCP assembly. I will discuss our latest work in defining HBV assembly mechanism and the opportunities they offer for anti-viral drug development.

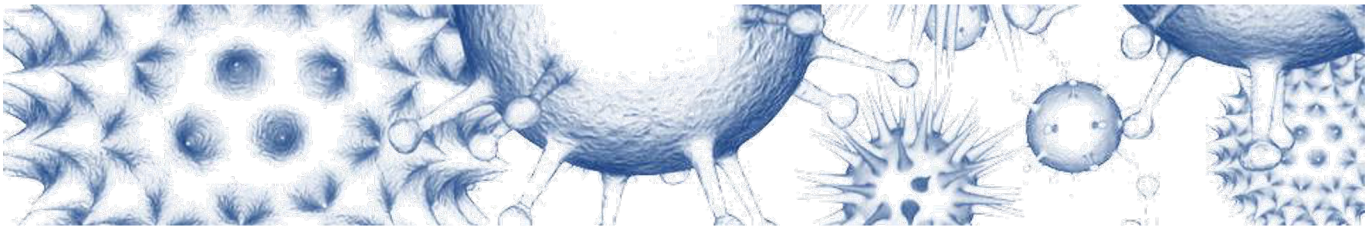


24 – INHIBITORS OF HIV-1 RT: EXPLORING NEW SCAFFOLDS FOR THE DUAL INHIBITION OF POLYMERASE AND RIBONUCLEASE ASSOCIATED FUNCTIONS

Alessia Onali[1], Rita Meleddu[1], Angela Corona[1], Simona Distinto[1], Filippo Cottiglia[1], Serenella Deplano[1], Lisa Sequeira[1], Daniela Secci[1], Erica Sanna[1], Francesca Esposito[1], Francesco Ortuso[2], Stefano Alcaro[2], Enzo Tramontano[1], and Elias Maccioni[1]

[1] Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato, Monserrato, 09042 Cagliari, Italy
[2] Dipartimento di Scienze della Salute, Università Magna Graecia di Catanzaro, Campus 'S. Venuta', Viale Europa, 88100 Catanzaro, Italy

HIV infection is still a serious public health problem and the current therapeutic protocol, today called with the acronym HAART (Highly Active Anti-Retroviral Therapy) consist in the combination of distinct anti-retroviral drugs that act on viral targets that play a key role in the different stages of the HIV life cycle. However, the combination of several drugs entails an increased risk of toxicity and a complex management of pharmacokinetic and metabolic interactions that could be reduced by the identification single therapeutic agents capable of simultaneously target multiple viral functions. So far we have explored different molecular entities able to simultaneously inhibit both associated functions of HIV reverse transcriptase. According to a defined pharmacophore different scaffolds have been studied and structure activity relationships harvested. Intriguingly, some of the newly designed compounds do exhibited the desired dual activity and will be discussed in this presentation.



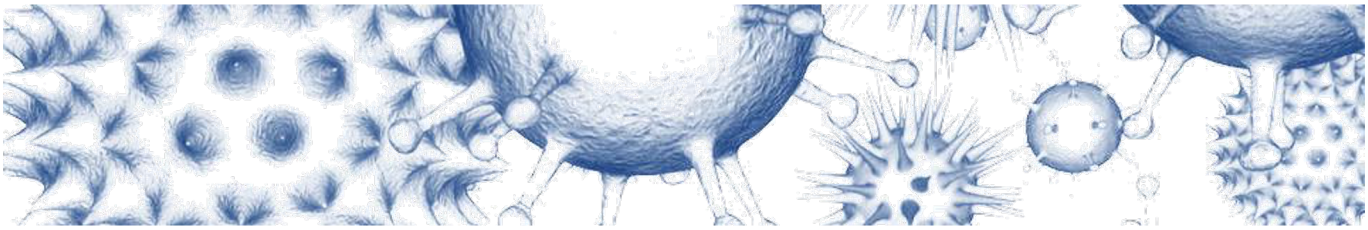
25 – SYNTHESIS OF FLEXIBLE NUCLEOS(T)IDE ANALOGUES OF REMDESIVIR IN BROAD SPECTURM ANTIVIRAL THERAPEUTICS

Charles D. Waters III[1], Evan Carlyle[1], Aren Vista[1], Prof. Katherine Seley-Radtke[1]

[1]Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore MD, USA.

Nucleos(t)ide analogues have a rich history as small molecule therapeutics used in treatments for numerous viruses as well as cancers. As such, there is a constant need to produce novel nucelos(t)ide analogues to help combat emerging and reemerging infectious diseases. The ongoing COVID-19 pandemic, caused by SARS-CoV-2, has further highlighted this need, as many preexisting and experimental nucleos(t)ide analogues have been pursued for repurposing for the treatment of SARS-CoV-2. One nucleotide prodrug that has shown antiviral potential is Remdesivir (RDV). RDV has shown antiviral activity against EBOV, SARS-1, and MERS and is an approved treatment for patients with SARS-CoV-2. However, RDV possess some minor drawbacks, such as poor oral bioavailability.

The Seley-Radtke lab has developed several nucleoside analogues that possess flexible purine base moieties, called Fleximers. The added flexibility endows these analogues with advantageous characteristics, as it allows for the nucleoside to adapt conformations that were previously unavailable to the rigid parent nucleoside, while remaining active. In some cases, newfound activity not seen in the parent compound can also be seen, as is with the case of a fleximer analogue of Acyclovir (ACV) produced by the lab. This analogue has displayed broad spectrum activity against Ebola, SARS, MERS, Dengue, and yellow fever, whereas ACV only exhibits activity against HSV. Herein we describe attempts to synthesize a flexible nucleoside of RDV in order to expand upon its antiviral potential and produce a broad-spectrum antiviral nucleoside analogue, and hopefully improve upon its oral bioavailability and pharmacokinetic profile.

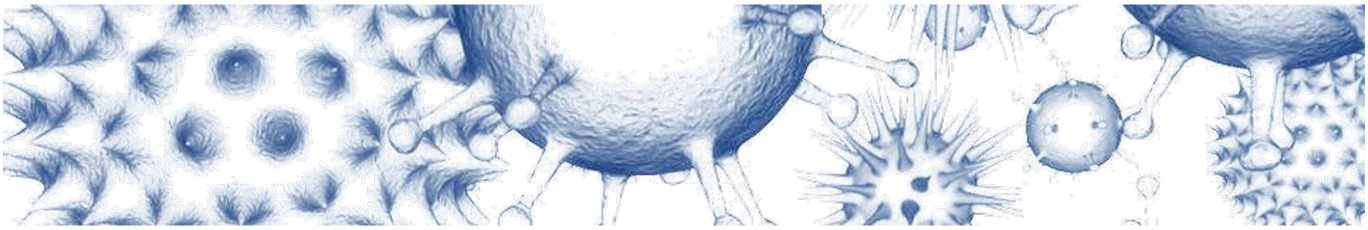


26 – HUMAN CYTOMEGALOVIRUS MEDIATES APOBEC3B RELOCALIZATION EARLY DURING INFECTION THROUGH A RIBONUCLEOTIDE REDUCTASE-INDEPENDENT MECHANISM

Elisa Fanunza[1,2,3,4], Sofia Moraes[1,2,3,4], Adam Cheng[1,2,3,4], Craig Bierle[3,5], Wade Bresnahan[3,6], Reuben Harris[1,2,3,4,7]

[1] Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota, USA.
[2] Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA.
[3] Institute for Molecular Virology, University of Minnesota, Minneapolis, Minnesota, USA.
[4] Center for Genome Engineering, University of Minnesota, Minneapolis, Minnesota, USA.
[5] Department of Pediatrics, Division of Pediatric Infectious Diseases and Immunology, University of Minnesota, Minneapolis, Minnesota, USA
[6] Department of Microbiology and Immunology, University of Minnesota, Minneapolis, MN, USA
[7] Howard Hughes Medical Institute, University of Minnesota, Minneapolis, Minnesota, USA

The APOBEC3 (A3) family of DNA cytosine deaminases represents an essential innate antiviral defense. A novel counter-restriction mechanism was uncovered for gamma-herpesviruses EBV and KSHV and alpha-herpesvirus HSV-1, which utilize the viral ribonucleotide reductase (RNR) large subunit to directly bind and relocalize A3B and A3A. Here, we asked whether this A3 relocalization mechanism is shared with the beta-herpesvirus HCMV. We found that A3B is relocalized from the nucleus to the cytoplasm upon HCMV infection of multiple cell types. However, the HCMV RNR, UL45, is not required for A3B relocalization demonstrating a fundamentally different molecular mechanism. The kinetics of A3B relocalization were rapid indicating involvement of an immediate early-early (IE-E) viral protein. To further test this hypothesis, infected cells were treated with cycloheximide (CHX), which restricts the expression of IE-E proteins, or with phosphonoacetic acid (PAA), which is a viral DNA synthesis inhibitor affecting expression of late proteins. We found that upon treatment with CHX, A3B relocalization was blocked, whereas treatment with PAA did not have any effect. Moreover, after infection with an UV-inactivated virus, A3B relocalization was reduced but not completely abolished. These results suggest that an IE-E protein potentially present in virions contributes to A3B relocalization.

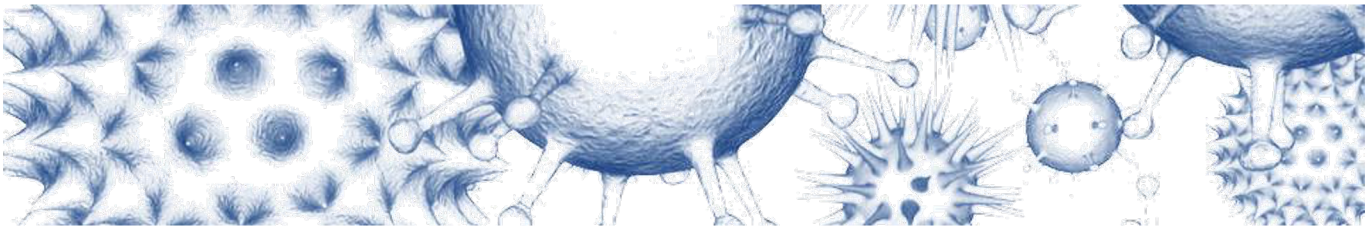


27 – CHARACTERIZATION OF VACCINE BREAKTHROUGH CASES EXHIBITED IN A COHORT OF CHILEAN ADULTS IMMUNIZED WITH AN INACTIVATED SARS-COV-2 VACCINE

Luisa F. Duarte[1,2], Nicolás MS Gálvez[1,2], Carolina Iturriaga[3], Felipe Melo-González[1,2], Jorge A Soto[1,2], Bárbara M Schultz[1,2], Marcela Urzúa[3], Liliana A. González[1,2], Yaneisi Vázquez[1,2], Mariana Ríos[1,2], Roslye V Berríos-Rojas[1,2], Daniela Rivera-Pérez[1,2], Daniela Moreno-Tapia[1,2], Gaspar A. Pacheco[1,2], Omar P Vallejos[1,2], Guillermo Hoppe-Elsholz[1,2], María S Navarrete[4], Álvaro Rojas[1,2], Rodrigo Fasce[5], Jorge Fernández[5], Judith Mora[5], Eugenio Ramírez[5], Gang Zeng[6], Weining Meng[6], José V González-Aramundiz[7], Pablo A González[1,2], Katia Abarca[1,3], Susan M Bueno[1,2], Alexis M Kalergis[1,2,8].

[1] Millennium Institute on Immunology and Immunotherapy.
[2] Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.
[3] Departamento de Enfermedades Infecciosas e Inmunología Pediátricas, División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.
[4] Departamento de Enfermedades Infecciosas del Adulto, División de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.
[5] Departamento de Laboratorio Biomédico, Instituto de Salud Pública de Chile.
[6] Sinovac Biotech, Beijing, China.
[7] Departamento de Farmacia, Facultad de Química y de Farmacia. Pontificia Universidad Católica de Chile, Santiago, Chile.
[8] Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.

Several vaccines have been approved for emergency use in the population to prevent infections by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and control the ongoing global pandemic. In Chile, about 80% of the immunized population have received CoronaVac, an inactivated SARS-CoV-2 vaccine that has shown effectiveness of 65.9% for symptomatic cases. Assessment of the immune response elicited upon vaccination and the follow-up after subsequent infections will provide valuable information to understand the possible causes behind vaccine breakthrough cases and to improve the approaches currently being used to enhance the protection in susceptible individuals in the future. We have characterized the humoral immune response elicited by seven mild, one moderate, and one severe vaccine breakthrough cases as part of our ongoing clinical trial (clinicaltrials.gov NCT04651790) by detecting circulating IgG antibodies specific against the RBD of the S1 protein of SARS-CoV-2 by ELISA, and the evaluation of their neutralizing capacities by a surrogate virus-neutralizing test and a conventional plaque-reduction neutralization test. Moreover, we evaluated the cellular immune response through ELISPOT assays using mega pools of peptides derived from the SARS-CoV-2 proteome. Our results did not show a similar immune response pattern among breakthrough cases correlated with the susceptibility to presenting a symptomatic SARS-CoV-2 infection. Still, the severity of the disease was related to older age and comorbidities that could impair the immune response induced upon immunization. In conclusion, CoronaVac is effective and vaccine breakthrough cases are mainly mild, and in those with moderate or severe disease it helps to prevent death.

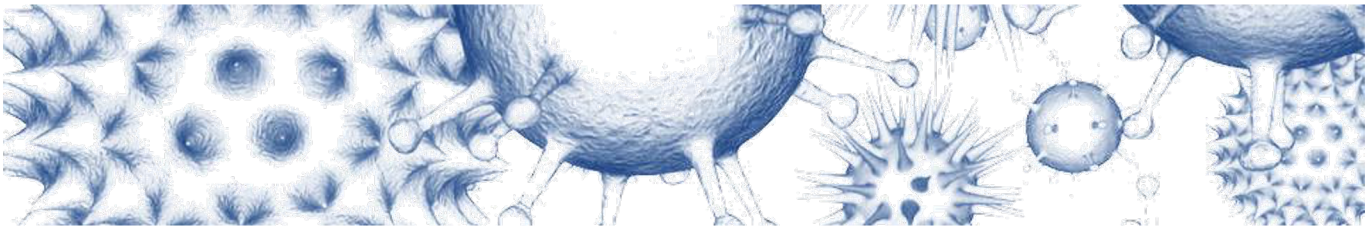


28 – COMPLEX INHIBITION CHARACTERISTICS OF HBV AND HIV RIBONUCLEASE H INHIBITORS AGAINST HUMAN RIBONUCLEASE H1

Nathan L Ponzar[1], Razia Tajwar[1], John E, Tavis[1]

[1]Saint Louis University School of Medicine, Dept. of Molecular Microbiology and Immunology

Many HIV and HBV ribonuclease H inhibitors also inhibit the homologous human RNase H1 (RNase H1). RNase H1 is necessary for mitochondrial replication and maintenance of genome integrity. Treatment courses for HBV and HIV with RNase H drugs will be long, so selectivity for the viral enzymes over the RNase H1 must be high. To aid development of selective antiviral compounds, we characterized binding and inhibition of RNase H1 by two α-Hydroxytropolone (αHT) RNaseH inhibitors. αHTs coordinate the two active site divalent metal ions in RNase H active sites. We measured the shift of the compounds' IC_{50}s in increasing Mg^{2+}. As expected, inhibition was Mg^{2+}-dependent, however, compound binding in the absence of substrate was not. This suggests a complex interplay of the enzyme and its three ligands. Steady-state inhibition data fit best a noncompetitive model ($p<0.0001$) in which V_{max} is suppressed but K_M is unchanged. In contrast, RNase H1 containing only the catalytically active domain without the substrate binding domain (HBD) fit best to a mixed inhibition model in which V_{max} is reduced and K_M is slightly increased ($p<0.0001$), indicating inhibition of substrate binding in the absence of the HBD. This indicates the HBD reduces sensitivity of human RNase H1 to inhibition of substrate binding by αHTs. These results suggest that existing αHT RNase H inhibitors will not prevent RNase H1 from engaging its natural substrates but rather slow its catalytic activity. Future studies will determine how these active-site binding compounds behave unexpectedly as inhibitors of predominantly noncompetitive character.

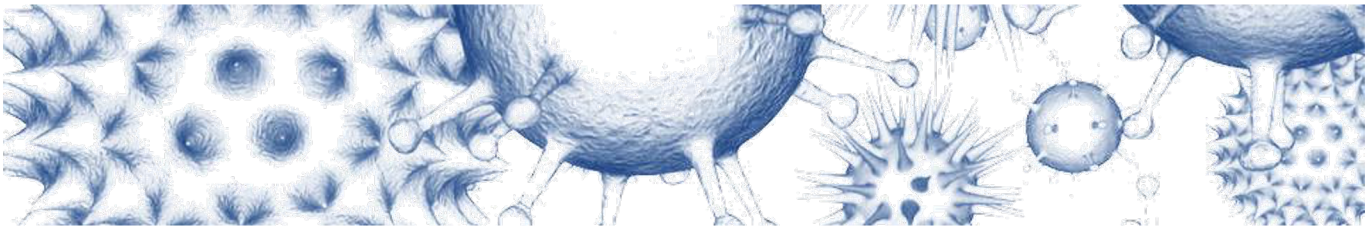


29 – IDENTIFICATION, COMPREHENSIVE CHARACTERIZATION AND COMPARATIVE GENOMICS OF THE HERV-K(HML8)INTEGRATIONS IN THE HUMAN GENOME

Sante Scognamiglio[1], Nicole Grandi[1], Maria Paola Pisano[1], Eleonora Pessiu[1], and Enzo Tramontano[1,2]

[1] Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato - SS554, 09042 Monserrato, Cagliari (Italy)
[2] Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche (CNR), 09042 - Monserrato, Cagliari (Italy)

Human Endogenous Retroviruses (HERVs) are major components of our genome and are currently highly investigated for their possible contribution to human physiopathology. However, the majority of HERV group are not well characterized. This is the case of the HML8 group, a member of the HERV-K superfamily that is still poorly accounted for its genomic distribution and expression in human tissues. In the present study, we identified 78 HML8 proviruses in human genome assembly hg38. We found that some chromosomes are significantly enriched (8 and X, p<0,005) for HML8 insertions, while others present fewer HML8 elements than expected (17 and 20). HML8 elements can be further classified into 3 phylogenetic clusters, associated with LTR types MER11A, MER11B, and MER11C. Comparative genomics showed that the diffusion of the group among primates has been limited to *Catarrhini* parvorder, being absent in older primates. Interestingly, the three phylogenetic clusters showed a different timing of colonization of primates' genome, with MER11A-associated proviruses that resulted significantly older. Accordingly, these elements constitute 80% of the integrations acquired from Rhesus Macaque being only occasionally introduced in subsequent lineages. In addition, several elements of the younger MER11B and MER11C clusters were found in the human genome sequence onlyleaving open the possibility of a tail of HML8 activity leading to human-specific integrations and/or duplication events. Finally, about 30% of the identified HML8 proviruses are co-localized within human genes, also within exons, being worth to be further studied for their possible impact on gene expression.

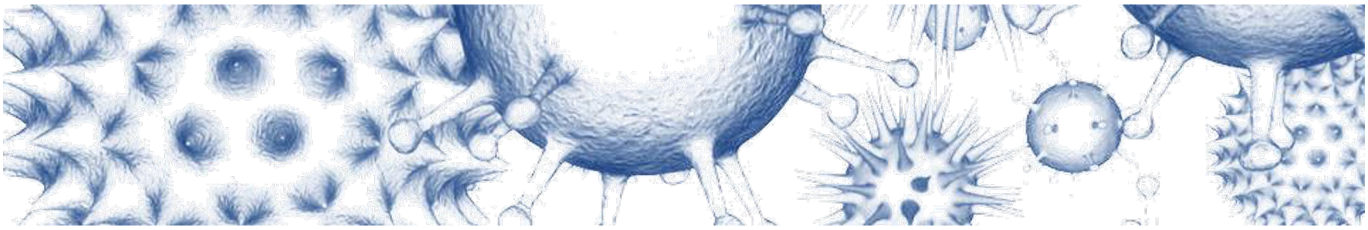


30 – MONOCLONAL HUMAN ANTIBODIES THAT RECOGNISE THE EXPOSED N AND C TERMINAL REGIONS OF THE OFTEN-OVERLOOKED SARS-CoV-2 ORF3A TRANSMEMBRANE PROTEIN

Tyng Hwey Tan[1,2], Carol Munro[2], Dora Edith[2], Andy Porter[1], Soumya Palliyil[1]

[1]Scottish Biologics Facility, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZP, UK
[2]Aberdeen Fungal Group School of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZP, UK

ORF3a has been identified as a viroporin of the virus and is known to be involved in various pathophysiological activities Including: disturbance of cellular calcium homeostasis, inflammasome activation, apoptosis induction and disruption of autophagy. ORF3a-targeting antibodies may specifically and favourably modulate these viroporin-dependent pathological activities. However, suitable viroporin-targeting antibodies are challenging to generate because of difficulties overcoming common technical challenges associated with generating antibodies to complex transmembrane proteins. Here we exploited a naïve human single-chain phage display library to isolate binders against carefully chosen ORF3a recombinant epitopes located towards the exposed N and C terminus of the protein. These binders were subjected to further characterisation using enzyme-linked immunosorbent assay and Biacore surface plasmon resonance analysis to identify high affinity binders. Their binding to full recombinant ORF3a protein was evaluated in western blot and fluorescent microscopy using ORF3a transfected cells. Co-localisation analysis was also performed to evaluate the “pairing potential” of the selected binders as possible alternative diagnostic or prognostic biomarkers for Covid-19 infections. Both ORF3a N- and C- termini epitope-specific monoclonal antibodies were identified in our study. Their therapeutic potential will now be further investigated in suitable *in vivo* models of disease. Whilst the linear nature of peptides might not always represent their native conformations in the context of full protein with carefully designed selection protocols, we have been successful in isolating anti-ORF3a binders capable or recognising that part of the transmembrane protein that is exposed on the “inside” or “outside” of the infected cell.

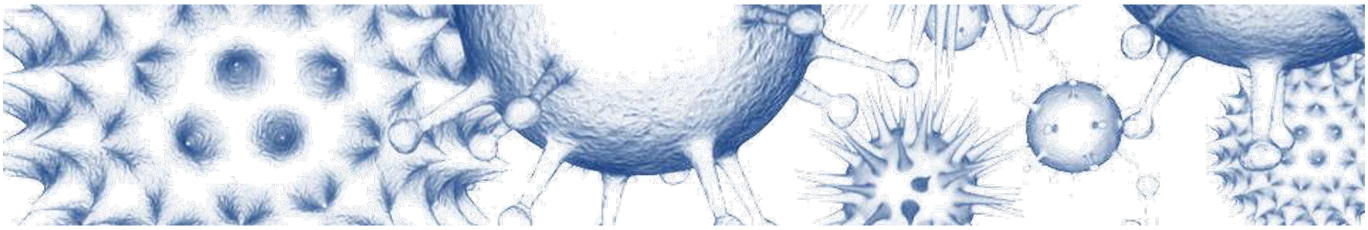


31 – GAIN-OF-FUNCTION ASSAY FOR SARS-CoV-2 M^{PRO} INHIBITION IN LIVING CELLS

Seyad Arad Moghadasi,[1,2,3] Jordan T. Becker,[1,2,3] Christopher Belica,[1,2,3] Chloe Wick,[1,2,3] William L. Brown,[1,2,3] and Reuben S. Harris[1,2,3,4]*

[1] Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota, USA, 55455
[2] Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA, 55455
[3] Institute for Molecular Virology, University of Minnesota, Minneapolis, Minnesota, USA, 55455
[4] Howard Hughes Medical Institute, University of Minnesota, Minneapolis, Minnesota, USA, 55455

The main protease, M^{pro}, of SARS-CoV-2 is required to cleave the viral polyprotein into precise functional units for virus replication and pathogenesis. Here we demonstrate a quantitative reporter for M^{pro} function in living cells, in which protease inhibition by genetic or chemical methods results in strong eGFP fluorescence or luminescence using firefly luciferase. This robust gain-of-function system readily distinguishes between inhibitor potencies and has been applied for high throughput drug screening and comparative testing of M^{pro} mutants. Using this assay we have compared different published inhibitors as well as identified new potential lead molecules. Furthermore, we have identified an actively circulating M^{pro} mutant that is more susceptible to inhibition by the FDA-approved hepatitis C protease inhibitor boceprevir. This novel assay will be an asset for expediting development of M^{pro} inhibitors for the treatment of SARS-CoV-2 as well as potential future zoonotic coronaviruses.



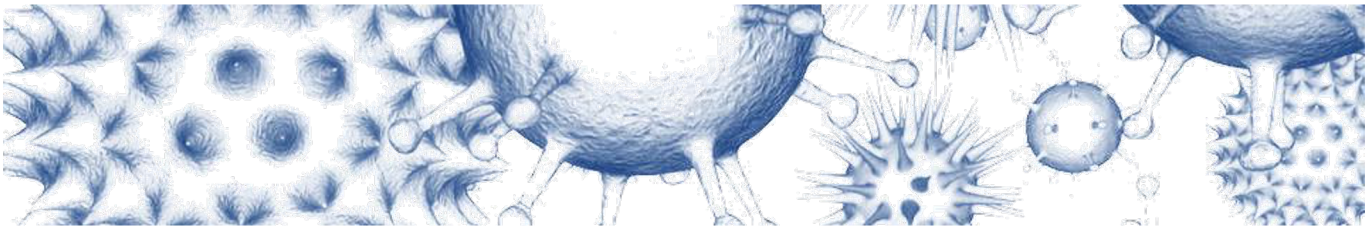
32 – OPTIMIZATION OF A PHENYLETHYNYL ANTHRANILIC ACID BASED DIHYDROOROTATE DEHYDROGENASE INHIBITOR SERIES FOR THE USE AS ANTIVIRAL AGENTS

Desiree Rijono[1], Nora Constanze Fohrmann[1], Chris Meier[1*]

[1]Organic Chemistry, Department of Chemistry, Faculty of Mathematics, Informatics and Natural Sciences, Universität Hamburg, Hamburg, Germany

Dihydroorotate dehydrogenase (DHODH) is a mitochondrial enzyme involved in the *de novo* pyrimidine synthesis pathway, leading to the formation of uridine monophosphate (UMP). Afterwards, UMP is converted to the other pyrimidine nucleotides needed for the biosynthesis of RNA and DNA. In resting cells, the demand for pyrimidines is covered by the *salvage* pathway, whereas fast proliferating cells, like virally infected cells, additionally depend on the *de novo* pyrimidine synthesis. The fact that 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 a suppression of the *de novo* biosynthesis of UMP and thus to reduced levels of pyrimidine nucleotides that primarily affect the replication of the viral genome. DHODH inhibitors function as host-acting antiviral agents that provide several advantages compared to inhibitors targeting unique viral enzymes, such as acting as broad-spectrum antivirals and provide a higher barrier to resistance.

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. Herein, we describe initial optimizations that were carried out on lead structures of the phenylethynyl 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.



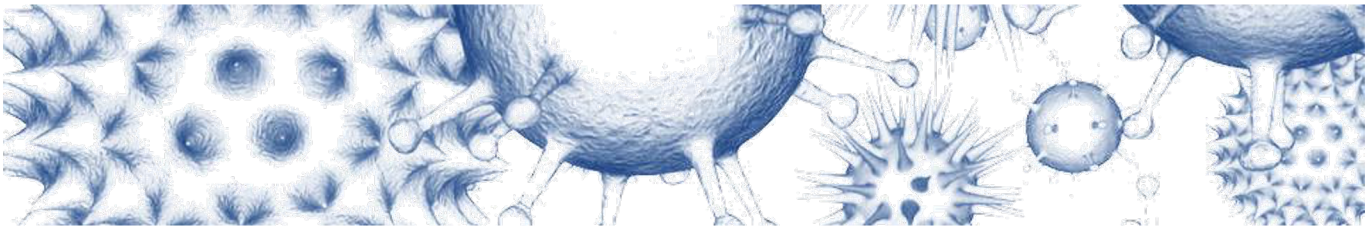
33 – SYNTHESIS OF MODIFIED T-1106-5´-TRIPHOSPHATES AS POTENTIAL INHIBITORS OF THE SARS-CoV-2 RNA POLYMERASE

Benedikt Ganter[1], Johanna Huchting[1], Bruno Canard[2], Chris Meier[1]

[1] Faculty of Sciences, Department of Chemistry, Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
[2] Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix-Marseille Université, UMR 7257, 13009 Marseille, France

The current global pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has clearly shown the urgent need for antiviral therapeutics. In the past, nucleoside analogues were used successfully for the treatment of several viral diseases by inhibiting the viral RNA-dependent-RNA-polymerase (RdRp). We have recently shown that the nucleoside analogue T-1106, that contains the non-fluoro-favipiravir base T-1105, is rapidly incorporated into the viral genome by the SARS-CoV-2 RdRp from its 5'-triphosphate form. The incorporation of T-1106 triggers C-to-U and G-to-A transition mutations, causing an antiviral effect through lethal mutagenesis.

The present study focusses on the synthesis of ribose-modified T-1106-5´-triphosphates with the aim of generating molecules with superior antiviral potency. Modifications were designed on the basis of known nucleoside and nucleotide analogue antivirals, such as sofosbuvir. After successful synthesis of ribose-modified T-1106s, the corresponding triphosphates were obtained in good yields following a modified Yoshikawa procedure which proved to be highly reproducible. Regarding viral polymerase inhibition, these T-1106 triphosphates are currently being evaluated. Compared to T-1106 and its triphosphate, such derivatives might show different mechanisms regarding polymerase inhibition, they may exhibit favourable selectivity and hence address much-discussed toxicity issues such as teratogenicity, and different *in vivo* properties could enhance antiviral potency.

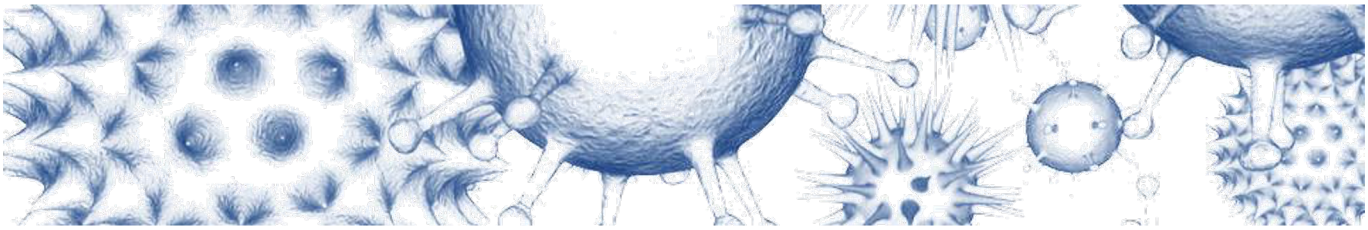


34 – DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF FLEX-ACYCLOVIR ANALOGUES AGAINST SARS-CoV-2

Joy E. Thames[1], Consuelo Correa-Sierra[2], Apurv Rege[1], Shuaishaui Liu[1], Chares Bieberich[1], Luis Schang[2], and Katherine L. Seley-Radtke[1]*

[1]Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore, MD, USA
[2]College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Since March of 2020, the world has been devastated by the SARS-CoV-2 pandemic. Even with vaccination efforts increasing, the pandemic continues to spread, and the search for direct acting antiviral drugs has intensified. In that regard, nucleos(t)ide analogues have a rich history as antivirals. One modification in nucleoside drug design that has proven successful, is the use of acyclic sugars, such as that found in Acyclovir (ACV), an FDA-approved drug for herpes simplex virus. Acyclic nucleosides lack the 2' and/or 3' carbons and the typical hydroxyl groups of the sugar moiety found in the naturally occurring nucleosides. For many years research in the Seley-Radtke group has focused on the development of novel nucleos(t)ide analogues known as "fleximers", which feature a "split" purine nucleobase, where a carbon-carbon single bond connects the pyrimidine and imidazole rings, thus introducing flexibility to the nucleobase scaffold. This flexibility endows the analogues with potent activity not seen for the corresponding rigid analogues. Combining the flex-nucleobase with the acyclic sugar of ACV produced a series of doubly flexible Flex-ACV analogues. These novel analogues have exhibited low micromolar to nanomolar levels of activity against SARS, MERS and human coronaviruses, as well as Ebola, Yellow Fever, Dengue and tickborne encephalitis, while ACV has no activity against those viruses. Following these observations, Flex-ACV analogues HP-083, HP-083-OAc, and HP-083-McG have now been screened against SARS-CoV-2 and COVID-19 *in vitro* and *in vivo*, as well as to undergo MTD and other pharmacokinetic studies. The results of those efforts are described herein.



35 – EXPLOITING A PROPRIETARY COMPOUND LIBRARY TO FIGHT SARS-CoV-2: IDENTIFICATION OF 2-PHENYLQUINOLINES WITH PAN-ANTICORONAVIRUS ACTIVITY

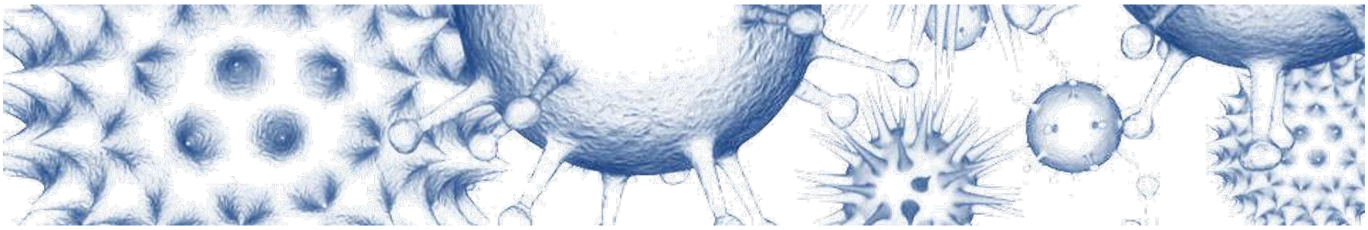
Maria Giulia Nizi[1], Leentje Persoons[2], Angela Corona[3], Tommaso Felicetti[1], Serena Massari[1], Giuseppe Manfroni[1], Laura Vangeel[2], Dirk Jochmans[2], Jessica Milia[3], Violetta Cecchetti[1], Johan Neyts[1], Stefano Sabatini[1],* Steven De Jonghe[2],* and Oriana Tabarrini[2]*

[1] Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
[2] KU Leuven, Rega Institute for Medical Research, Department of Microbiology, Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Herestraat 43, box 1043, 3000 Leuven, Belgium
[3] Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato (CA), Italy.

Over the years, our laboratory has worked on the design and synthesis of biologically active compounds resulting in an in-house library that mainly consisted of antiviral small heterocyclic molecules. The strategy to evaluate previously developed compounds for a new biologically relevant activity could most plausible led to identify innovative chemotypes for a certain pathology.

Thus, we selected a first set of about 100 compounds endowed with the most diverse structures and biological activities and tested them for antiviral activity against SARS-CoV-2. A first compound, based on a 2-phenylquinoline (2-PhQ) scaffold and belonging to a large series of derivatives developed as NorA *S. aureus* efflux pump inhibitors, was identified showing EC50 and CC50 values of 6 and 18 μM, respectively. The successive selection, design and synthesis of an enlarged series of analogues led to compounds that maintained low μM antiviral activity, while lacking cytotoxicity at the highest concentration of 100 μM. The most promising compounds were even more active against the human coronaviruses HCoV-229E and HCoV-OC43, with EC50 values ranging from 0.2 to 9.4 μM. These data highlight the 2-PhQ scaffold as particularly suitable for the discovery of *pan*-anticoronavirus agents with the potential to be also active against future pandemics.

Quinoline-based compounds are endowed with a wide variety of pharmacological activities. The anti-SARS-CoV-2 activity of chloroquine is mainly resulting from inhibition of autophagy by impairing autophagosome fusion with lysosomes. Although our compounds share with chloroquine the core structure and the antiviral profile, the inhibition of autophagy by 2-PhQ in VeroE6 cells was less pronounced than by chloroquine. Thus, additional and/or alternative targets, such as the two viral proteases, the RNA-dependent RNA polymerase and the helicase are currently under exploration.



36 – THE US12 PROTEIN FAMILY OF HUMAN CYTOMEGALOVIRUS: FROM FUNCTIONS TO ANTIVIRAL INTERVENTION

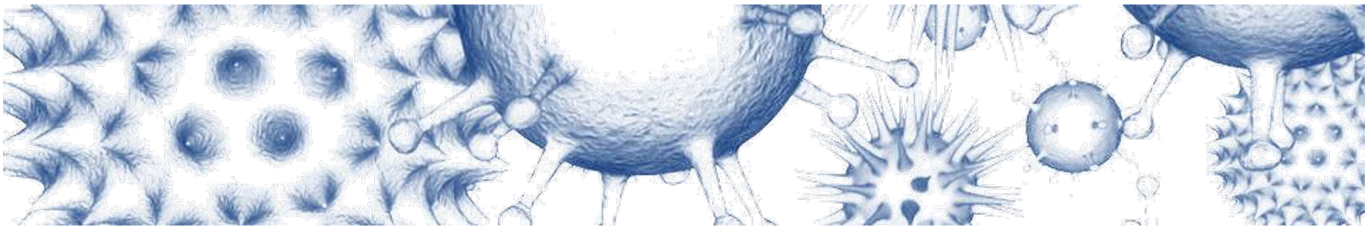
Giorgio Gribaudo

Department of Life Sciences and Systems Biology, University of Torino, 10123 Torino, Italy
(giorgio.gribaudo@unito.it)

During coevolution with its host, the Human Cytomegalovirus (HCMV) has invested a large part of its protein coding potential to ensure the regulation of viral spread, persistence and reactivation, as well as the modulation of intrinsic, innate and acquired host immune responses. Among the viral genes involved, the US12 gene family includes a set of 10 contiguous tandemly arranged genes (US12 to US21) that are not strictly required for the in vitro HCMV replication in fibroblasts, and therefore classified as nonessential. The identification of putative seven transmembrane hydrophobic domains in each of the US12 ORFs then predicts a common structural framework that associates these proteins to cellular membranes. However, despite the likely evolutionary importance of the US12 genes to HCMV biology, only a few functions have been associated with the family to date.

By means of a functional reverse genetic analysis of the whole gene family, we confirmed that inactivation of individual US12 gene members in the context of the low-passage HCMV strain TR genome did not significantly impact on productive viral replication in fibroblasts. Nevertheless, inactivation of the US12, US16, US18, US20, or US21 genes was observed to affect TR growth in other cell types, such as endothelial and epithelial cells, thus suggesting roles of these US12 genes in contributing to efficient HCMV replication in cells other than fibroblasts. Starting from this observation, we have deepened the functional characterization of these five US12 proteins and the mechanisms by which they influence different aspects of virus-host interaction, such as the virus cell tropism by tuning the composition of virion envelope and tegument, the evasion of NK cell recognition of infected cells by downregulating ligands of NK activating receptors, and the intracellular Ca^{2+} homeostasis to counteract apoptosis and regulate cell migration and adhesion. Noteworthy, the Ca^{2+}-conducting function of the US12 and US21 viroporins could make these US12 proteins potential targets for identification of small molecule blockers as antiviral agents.

Together, these findings indicate that the US12 proteins are biologically active and contribute in various ways to HCMV replication and pathogenesis, and that their structures and functions could be exploited to select antiviral molecules.

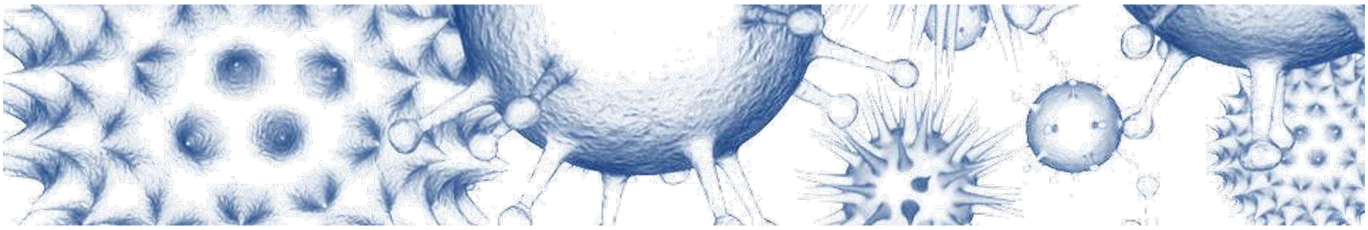


37 – DEVELOPMENT OF FUSION INHIBITORY PEPTIDES AGAINST AIRBORNE VIRAL INFECTIONS, MEASLES AND COVID-19

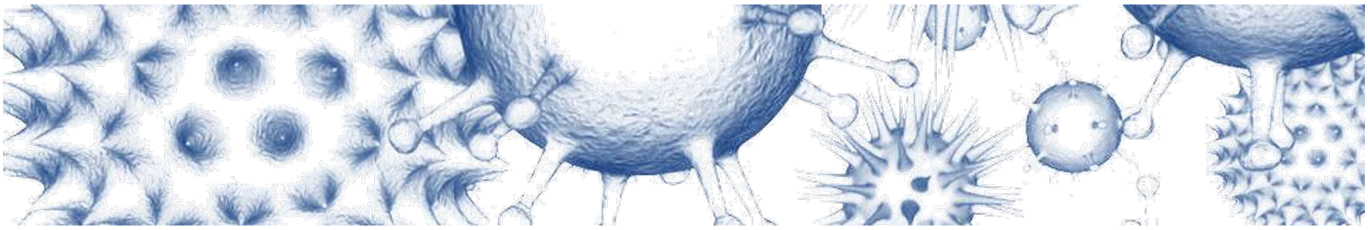
Branka Horvat

International Center for Infectiology Research-CIRI, Lyon, France

Airborne infection is transmitted through small aerosolised particles suspended in the air, and is responsible for the transmission of many infectious diseases of the considerable importance in both human and veterinary medicine. Although vaccination is supposed to be the best prevention for these infectious diseases, vaccines are not always available, particularly for new emerging pathogens and in some cases not sufficient in the prevention of new outbreaks. For an enveloped virus it is possible to identify candidate lead molecules directly from the genetic information, in the form of peptides corresponding to key domains of the viral fusion machinery. These peptides could specifically block viral fusion and consequently inhibit viral infection. Recent results have demonstrated that cholesterol tagged peptides provide highly potent fusion inhibitors with prolonged circulatory half-life *in vivo*. We focus on two important human pathogens: Measles virus (MeV) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). MeV is highly contagious virus which remains the leading cause of death among vaccine-preventable diseases and continues to resurge worldwide. SARS-CoV-2 is the etiological agent of the coronavirus disease 2019 (COVID-19) which is responsible for the current pandemic. Both viruses infect target cells by coordinated action of viral envelope glycoproteins. After binding of MeV hemagglutinin to the cell receptor, MV fusion protein initiates the virus-cell fusion, leading to the cell infection. We have developed the lipopeptide HRC4, capable of inhibiting MeV fusion and preventing measles infection in rodent models. We have further advanced this approach by administration of aerosolized peptides capable of inhibiting respiratory MeV infection in non-human primates, cynomolgus monkeys. We have developed an inhalation strategy using a customized nebulizer with the specific mesh size and interface ensuring a functionality of nebulized particles and their distribution in respiratory tract. Nebulization of HRC4 efficiently prevented MeV infection in monkeys, leading to the complete absence of MeV-RNA, MeV-infected cells and MeV-specific humoral response in treated animals housed separately from untreated. These results open new perspectives for antiviral prevention strategy against measles as well as the other airborne viruses, including SARS-CoV-2. Entry of SARS-CoV-2 into the host cells is mediated by ACE-2 receptor, which is a component of the angiotensin-regulating system. This virus binds ACE2 via its envelope Spike (S) glycoprotein, leading to the virus-cell fusion and consequent SARS-CoV-2 entry and replication. SARS-CoV-2 S is a homotrimer in which each monomer contains 2 subunits, S1 and S2. S2 is responsible for the fusion and presents two heptad repeat domains (HR) in N and C amino termini. The interaction between these domains (HRC and HRN) is critical for the membrane fusion. We have analyzed the capacity of fusion inhibitory peptides, derived from the heptad repeat domains in C-amino terminus (HRC) of Spike, to inhibit SARS-CoV-2 infection in a murine model, both *ex vivo* and *in vivo*.



We observed that the peptide causes significant inhibition of SARS-CoV-2 infection in organotypic cultures prepared from lungs of mice expressing human ACE2. *In vivo*, while SARS-CoV-2 is provoking 100% lethal infection within 10 days post-infection in K18-hACE2 mice, intranasal administration of peptides reduced the body-weight loss and protected between 80% to 100% of mice from the SARS-CoV-2-induced lethality. These results were associated with high decrease of viral load in lungs compared to the mock-treated group. These findings indicate that fusion inhibitory peptides highly reduce the clinical impact of SARS-CoV-2 infection in the animal model, thus providing a proof of concept for a new complementary approach of antiviral prophylaxis to be developed as part of the global effort against the current SARS-CoV-2 pandemic. This antiviral strategy may form the basis for an efficacious and timely emergency response, immediately following identification of potentially dangerous new virus using similar fusion mechanism for viral entry.



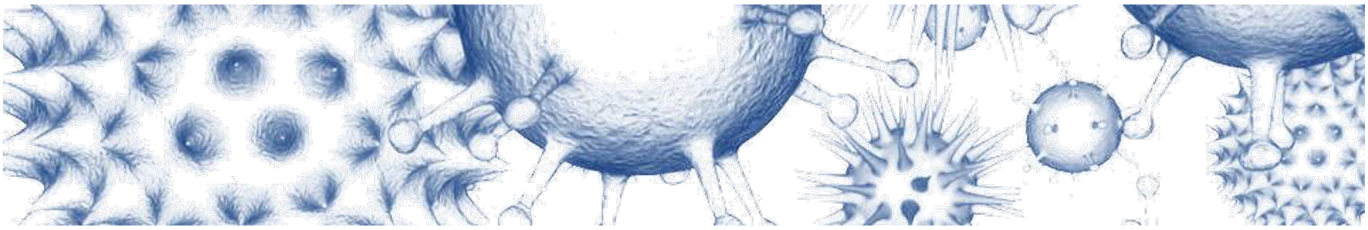
38 – HIV-1 REPLICATION: FIDELITY, DRUG RESISTANCE AND UNEXPLOITED ANTIVIRAL TARGETS

Luis Menéndez-Arias

Centro de Biología Molecular "Severo Ochoa" (Consejo Superior de Investigaciones Científicas & Universidad Autónoma de Madrid), Madrid, Spain

Reverse transcriptases (RTs) are nucleic acid polymerases that synthesize DNA using RNA or DNA as templates, while degrading RNA when forming RNA/DNA hybrids (RNase H activity). RTs play a pivotal role in the replication of several virus families, most notably *Retroviridae* and *Hepadnaviridae*. These enzymes are devoid of proof-reading activity and contribute to the large variability observed in HIV, with intrinsic error rates of 10^{-4} to 10^{-5}. HIV-1 RT inhibitors acting on its DNA polymerase activity constitute the backbone of currently prescribed antiretroviral therapies. Although nucleoside analogues are effective against HIV-1 and HIV-2 strains, HIV-2 shows natural resistance to efavirenz and other non-nucleoside RT inhibitors (NNRTIs). In HIV-1, development of resistance to nucleoside inhibitors can be achieved through the acquisition of mutations affecting residues of the nucleotide binding site (e.g. K65R, M184V) and/or substitutions that facilitate the excision of the drug from the 3´ end of the primer (e.g. M41L, T215Y and other thymidine analogue resistance mutations). The impact of the excision pathway in HIV-2 is negligible in comparison with HIV-1 due to amino acid differences found at the β3-β4 hairpin loop of their RT (specifically at residues 73 and 75).

The increased prevalence of drug resistance in some regions of the world anticipates the need of novel antiretroviral drugs targeting resistant viruses. We will review our efforts to understand RNase H function in reverse transcription and how RNase H mutational inactivation affects DNA-dependent DNA synthesis fidelity, template switching and strand displacement. In addition, we will discuss our recent findings on the characterization of novel active site RNase H inhibitors, including coumarin derivatives with dual inhibitory activity against RT DNA polymerase and RNase H. Some of these compounds were found to be potent inhibitors of the HIV-1 RT's RNA-directed strand displacement activity.

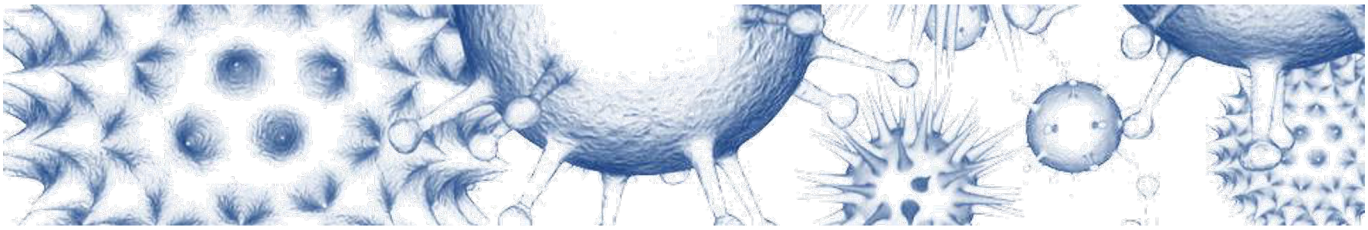


39 – A JOURNEY FROM HCV PROTEASE TO SARS-CoV2- 3CLP PROTEASE INHIBITORS IN CLINICAL TRIAL: CHALLENGES AND OPPORTUNITIES

Vincenzo Summa

Department of Pharmacy, University of Naples "Federico II", via D. Montesano 49, 80131 Napoli, Italy.

The ongoing COVID-19 pandemic led to an unprecedented global scientific effort to identify effective vaccines and small molecules therapeutics. The global effort culminated in the approval of several vaccines and one drug to fight the pandemic. The first attempts to identify effective drugs against the SARS-CoV-2 virus involved drug repurposing approaches, leading to the approval of Remdesivir, the first Nucleoside Prodrug acting as a polymerase inhibitor. This drug was originally identified as HCV and Ebola polymerase inhibitor and developed up to Phase III. On the contrary, the same approach failed to repurpose other previously reported protease inhibitors as selective inhibitors of SARS-Cov-2 3CL protease, one of the main targets of the virus. Consequently, the compounds which are currently under clinical evaluation were developed mostly by rational design, capitalizing on all the information previously acquired for other viral and host proteases, in particular HCV protease inhibitors. The presentation will focus on the different medicinal chemistry strategies applied for the identification of potent selective and safe new agents currently in clinical trials.



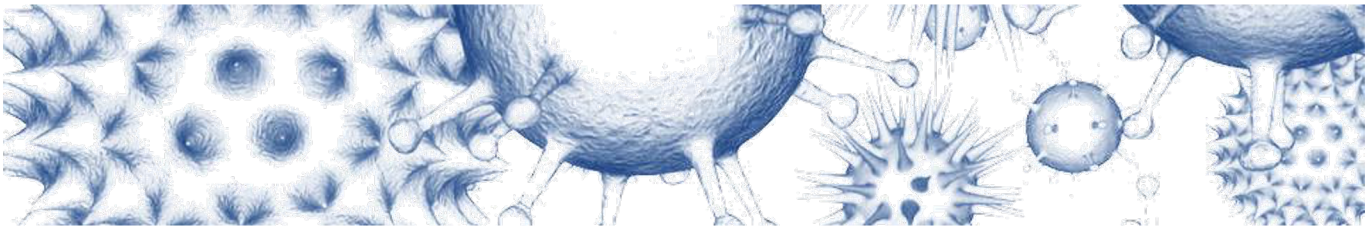
40 – ANTIVIRALS, A LOT HAS BEEN ACHIEVED, YET A LONG WAY TO GO

Johan Neyts

Laboratory of Virology, Rega Institute for Medical Research, Department of Microbiology & Immunology, University of Leuven (KU Leuven)
Johan.neyts@kuleuven.be

Today, small molecule antiviral drugs are available for the treatment of infections with herpesviruses, HIV, HBV and HCV as well as with influenza viruses. Yet, for many other viruses that cause life-threatening infections and many of which are considered emerging and/or neglected pathogens, there are no drugs available. These are needed for the treatment of infections with existing viruses but also in the context of epidemic and pandemic preparedness. I will discuss our efforts to develop potent antivirals against -of course – SARS-Cov2 and other coronaviruses, but also flaviviruses (such as dengue), alphaviruses (such as chikungunya) and enteroviruses. Several excellent molecular targets for the selective inhibition of viral replication (and that have remained largely unexplored) have been identified, such as the non-structural protein NS4B of flaviviruses, the capping machinery of alphaviruses and the 2C helicase of enteroviruses.

www.antivirals.be ; www.twitter.com/neytsvirology ; www.twitter.com/neyts_johan

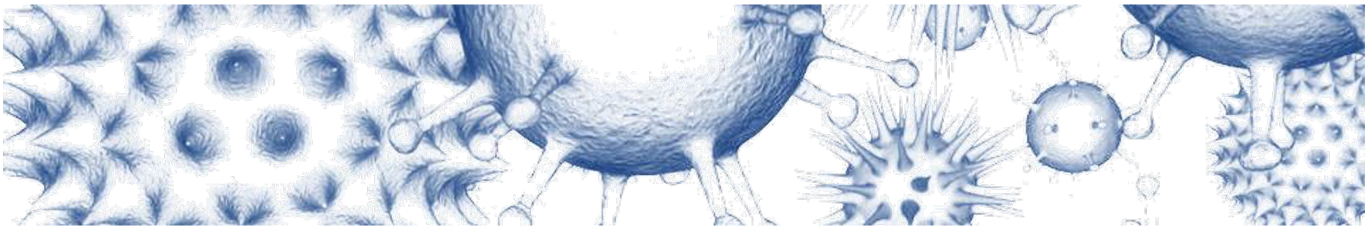


41 – DEVELOPING NOVEL VIRUS-LIKE AGENTS TO COMBAT VIRAL, BACTERIAL, AND OTHER PATHOGENS OF TREES TO PREVENT A FUTURE WITHOUT CITRUS, CHOCOLATE, GRAPEVINES AND OLIVES

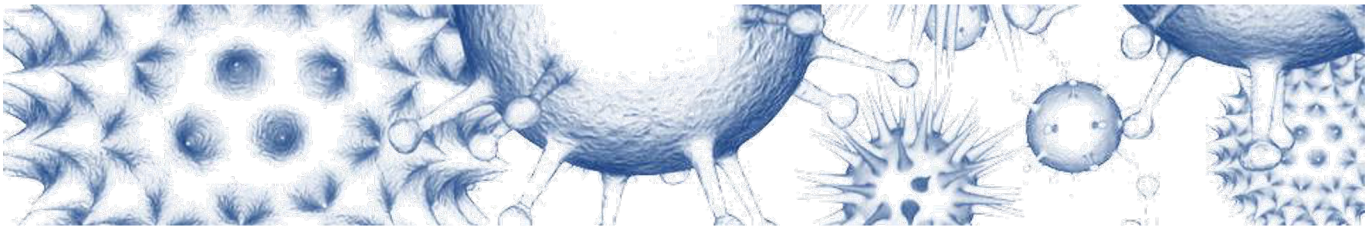
Anne Simon

University of Maryland College Park
Silvec Biologics

Imagine for a moment, a world without citrus, grapes, olives and chocolate. While not as impactful as human disease pandemics, pandemics caused by bacteria, fungi and viruses affecting long lived trees and vines have already begun to significantly impact the economies of many nations and the quality of life of people everywhere. It is estimated that in 10 years, citrus and cacao trees will not be viable outside of greenhouses, making the fruit prohibitively expensive for all but the wealthiest. Pathogens causing the most serious (deadly) diseases are those that invade the vascular system of plants, the veins through which sugar and water flow from source to sink. Unlike the many treatments and vaccines developed for human/animal pathogens, there are NO effective treatments or vaccines for any of these plant pathogens due to the difficulty in targeting vascular pathogens in a cost-effective manner. Although trees and vines can be generated that are resistant to pathogens, the genetic modifications necessary for resistance are prohibited in many countries, particularly those in Europe, requiring other approaches to keep the trees and vines alive. We have discovered a novel, virus-like replicon that is able to replicate and move independently throughout the vascular system of plants in a manner that differs from movement of plant viruses and which is nearly always associated with no discernable symptoms. In the absence of encoded movement proteins, this RNA, called citrus yellow vein-associated RNA or CYVaV, uses an ancient, highly conserved host RNA-binding protein for movement, likely by forming an RNase-resistant “packet” that requires one or more specific elements in the CYVaV 3’ terminal 300 nt. Unlike virtually all other plant viruses, CYVaV does not encode silencing suppressors, which are normally necessary to disable the endogenous RNA silencing pathways in the plant (a major component of the plant immune system). This is likely because translation of CYVaV uses a highly efficient -1 ribosomal frameshifting mechanism to bypass the first stop codon (30% compared with the normal average of 2-5%) to generate a high level of RNA-dependent RNA polymerase for genome replication. The goal of our research is to convert this 2.7 kb single-stranded, positive sense, independently mobile (i)RNA into a vector to deliver 21-24 nt siRNAs into the vascular system of trees and vines that can target bacterial, fungal and viral pathogens for the life of the plant. We have determined the secondary structure of CYVaV and found areas in the 3’ UTR that are amenable to small >40 nt inserts. Surprisingly, even hairpin inserts can have significant effects on structure throughout the iRNA. When coupled with reduced translation and/or ribosomal frameshifting, the inserts are unstable. Fortunately, we have discovered ways to stabilize the inserts and increase translation.



When producing siRNAs targeting a major citrus virus in a laboratory model plant, CYVaV can eliminate infectivity of this virus. Targeting of the highly pathogenic bacteria *Xylella fastidiosa* (the one killing the century old olive trees in Italy) using a different virus vector in our model plant can eliminate pathogenesis of this highly destructive bacterium. Since CYVaV should have an extensive host-range, it may be usable as a near universal vector to control diseases that are currently killing millions of trees and vines each year.

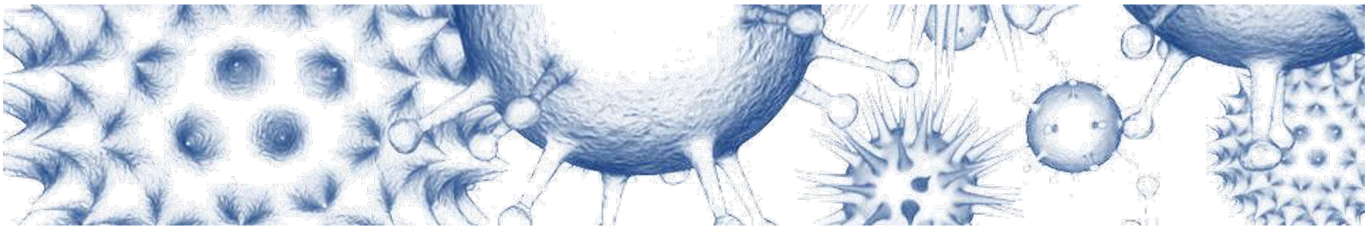


42 – DIRECT VISUALIZATION OF INDIVIDUAL RNA MOLECULES AND MAPPING THE RNA CONFORMATIONAL SPACE IN SOLUTION

Yun-Xing Wang

Protein-Nucleic Acid Interaction Section, Center for Structural Biology, National Cancer Institute, National Institutes of Healthy, Maryland,USA

For proteins, we are so used to the idea that given a primary amino acid sequence, there is a corresponding secondary structure and a cognate conformation. This idea is reinforced by the recent rapid development of predicting protein structures based on primary sequences. This notion implies that there is a “one-to-one” relationship between a primary sequence and the final three-dimensional (3D) structure. This “one-to-one” relationship has influenced our thinking about RNA for the past several decades, ever since the first 3D structure of tRNAphe was determined by Alex Rich in 1971. From then, whenever a piece of a new biologically important RNA is discovered, the first question that people always tend to ask is what it looks like in 3D after quickly learning its primary sequence and possible secondary structure. The answer to this primordial question is more than often disappointing: the 3D structure of the beloved RNA could not be determined! Most of the interesting RNAs can neither be crystallized for crystallography nor “visualized” under the latest powerful cryo-electron microscopes. This lecture is to illustrate why RNA structure determination is challenging and often futile and how we should reconsider the popular “one-to-one” relationship.

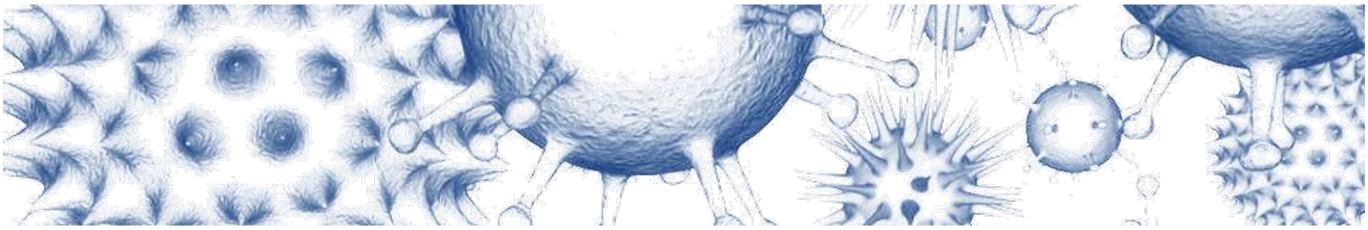


43 – IDENTIFYING NEW ANTIVIRALS, RESISTANCE AND HOST FACTORS

Josè Estè Araque

UIC, Barcelona, Spain

Most antivirals in development and those used in the clinic target viral enzymes, providing drug specificity and reduced unwanted effects. Viruses may develop resistance by acquiring mutations in the target gene, leading to treatment failure.
As internal parasites, viruses require the host machinery to replicate. Essential host factors for viral replication are amenable for drug development and those factors that are common to a variety of viruses may offer the unmet possibility of developing therapeutics with broad-spectrum antiviral activity. Additionally, it has been hypothesized that development of resistance may be avoided by drugs targeting host proteins. A number of viruses target critical cell cycle regulators to achieve cellular conditions required for viral replication. Cell cycle control may offer a number of targets for antiviral drug development. We will review the potential use of host factors for antiviral drug development, how drugs provide insight into the mechanisms of virus replication, and disease, and how viruses may escape the activity of drugs targeting host factors.

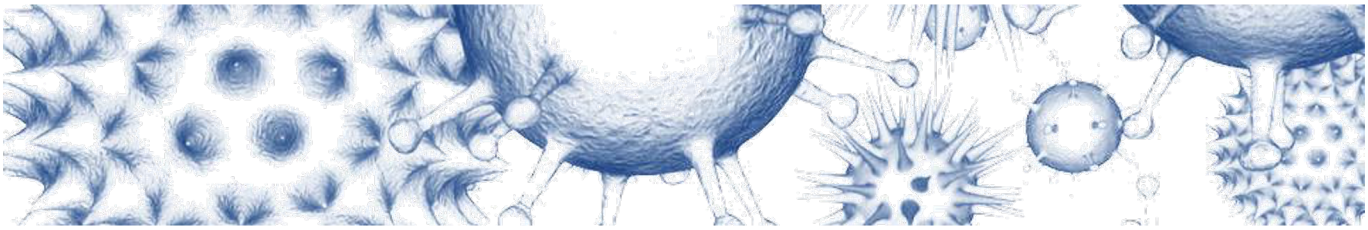


44 – ANTISENSE OLIGONUCLEOTIDES FOR THE CONTROL OF GENE EXPRESSION IN DISEASE AND INFECTION

Michelle L. Hastings

The Center for Genetic Diseases, Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL USA

Antisense oligonucleotides (ASOs) are synthetic nucleotides or nucleotide analogues that function by base-pairing to a specific targeted sequence. The specific base-pairing of these short, antisense nucleic acids to the targeted RNA results in degradation or modification of the RNA, and in this way, ASOs can control gene expression. ASOs have achieved clinical success in the treatment of human diseases and pathological conditions. Given this success, ASOs are widely regarded as a powerful therapeutic platform. We are using antisense technology to develop therapeutics for a number of neurodegenerative and neurosensory disorders such as Usher syndrome, Batten disease and Alzheimer's disease. ASOs are also being used in anti-viral approaches to directly down-regulate viral RNA or to control expression of endogenous genes required for viral infection. The use of antisense technology for the treatment of disease and their emerging application as anti-viral agents will be discussed.



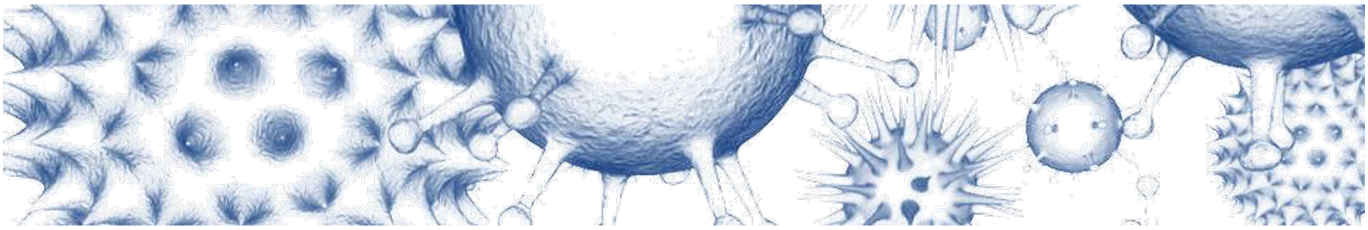
45 – ANIMAL MODELS FOR THE DEVELOPMENT OF ANTIVIRAL AGENTS AGAINST HEPATITIS B VIRUS (HBV)

Luca G. Guidotti

Deputy Scientific Director, Ospedale San Raffaele (OSR)
Full Professor of Pathology, Vita-Salute San Raffaele University, Medical School
Head, Immunopathology Unit, OSR
Via Olgettina n. 58, 20132 Milan, ITALY

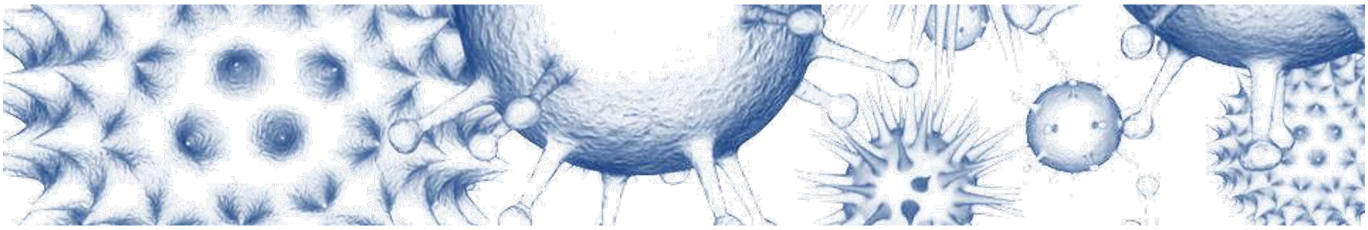
Hepatitis B virus (HBV) is the prototypic member of the Hepadnaviridae family that in humans causes acute or chronic liver infections. Chronic hepatitis B (CHB) often progresses to the development of complications like cirrhosis and hepatocellular carcinoma (HCC), which kill each year almost one million people worldwide. HBV is considered a noncytopathic virus and, therefore, immune related events are thought to play an important role in the pathogenesis and outcome of these infections. Therapy for CHB patients is limited to few antiviral drugs (nucleos(t)ide analogues or NUCs) that suppress viral replication but cannot eradicate HBV from the liver, requiring costly lifelong treatments. Moreover, CHB patients remain at risk of developing HCC despite years of NUC therapy. As such, there is a pressing need to find new strategies attaining a definitive cure for CHB. The experimental approaches to discovery and development of antiviral agents have been difficult because the host range of this virus is limited to humans and chimpanzees, and because of the lack of small animal models that are readily susceptible to HBV infection. Nonetheless, a great deal of information has been obtained in chimpanzees infected by HBV and woodchucks or ducks infected by related viruses (e.g. the woodchuck hepatitis virus, WHV and the duck hepatitis virus, DHBV). Additional insight has been gained from multidisciplinary studies in transgenic or chimeric/humanized mouse models that replicate the viral life cycles to varying degrees. A summary of the contributions that these animal models have given to the development of anti-HBV agents will be provided.

Participant contacts



Speakers

First name	Second name	Affiliation	e-mail
Graciela	Andrei	K.U. Leuven	graciela.andrei@rega.kuleuven.be
Andrea	Brancale	Cardiff University	brancalea@cardiff.ac.uk
Roberto	Di Santo	Università di Roma La Sapienza	roberto.disanto@uniroma1.it
Josè	Estè Araque	CienciaTraducida	jaeste@cienciatraducida.com
Giorgio	Gribaudo	Università di Torino	giorgio.gribaudo@unito.it
Luca	Guidotti	Vita-Salute San Raffaele University	guidotti.luca@hsr.it
Reuben	Harris	HHMI and University of Minnesota	rsh@umn.edu
Michelle	Hastings	Rosalind Franklin University	michelle.hastings@rosalindfranklin.edu
Branka	Horvat	International Center for Infectiology Research	branka.horvat@inserm.fr
Akiko	Iwasaki	Yale School of Medicine	akiko.iwasaki@yale.edu
Matteo	Iannacone	Vita-Salute San Raffaele University	iannacone.matteo@hsr.it
Jason	McLellan	University of Texas Austin	jmclellan@austin.utexas.edu
Luis	Menendez- Arias	Centro de Biología Molecular Severo Ochoa	lmenendez@cbm.csic.es
Ali	Mirazimi	Karolinska Institutet	ali.mirazimi@ki.se
Johan	Neyts	K.U. Leuven	Johan.Neyts@kuleuven.be
St. Patrick	Reid	Omaha University	patrick.reid@unmc.edu
Katherine	Seley-Radtke	University of Maryland	kseley@umbc.edu
Anne	Simon	University of Maryland	simona@umd.edu
Peter	Stockley	University of Leeds	P.G.Stockley@leeds.ac.uk
Vincenzo	Summa	University Napoli Federico II	vincenzo.summa@unina.it
Yun-Xing	Wang	National Cancer Institute	wangyunx@mail.nih.gov



Attendants

First name	Second name	Affiliation	e-mail
Seyad	Arad Moghadasi	University of Minnesota	Mogha019@umn.edu
Annalaura	Brai	University of Siena	annalaura.brai@unisi.it
Rolando	Cannalire	Università di Napoli Federico II	rolando.cannalire@unina.it
Loredana	Cappellacci	University of Camerino	loredana.cappellacci@unicam.it
Saili	Chabukswar	University of Cagliari	sailichabukswar03@gmail.com
Luisa Fernanda	Duarte Peñaloza	Pontificia Universidad Católica de Chile	lfduarte@uc.cl
Francesca	Esposito	University of Cagliari	francescaesposito@unica.it
Elisa	Fanunza	University of Minnesota	fanun001@umn.edu
Benedikt	Ganter	University of Hamburg	benedikt.ganter@chemie.uni-hamburg.de
Nicole	Grandi	University of Cagliari	nicole.grandi@unica.it j
Emmanuel	Heilmann	Medical University Innsbruck	Emmanuel.heilmann@i-med.ac.at
Christianna	Kutz	University of Maryland	ckutz1@umbc.edu
Saverio	Lemme	MIMIT – Tor Vergata	saverio.lemme@alumni.uniroma2.eu
Francesca	Miglioli	University of Parma	francesca.miglioli@unipr.it
Jessica	Milia	University of Cagliari	jessicamilia47@gmail.com
Maria Giulia	Nizi	Università di Perugia	mariagiulia.nizi@libero.it
Lia-Raluca	Olari	Ulm University	lia-raluca.olari@uni-ulm.de
Alessia	Onali	University of Cagliari	aleonali95@gmail.com
Selina	Pasquero	University of Turin	Selina.pasquero@unito.it
Annalaura	Paulis	University of Cagliari	annalaurapaulis@hotmail.it
Anthi	Petrou	School of Pharmacy, Aristotle University of Thessaloniki	anthi.petrou.thessaloniki1@gmail.com
Nathan	Ponzar	Saint Louis University	Nathan.ponzar@slu.edu
Desiree	Rijono	University of Hamburg	desiree.rijono@chemie.uni-hamburg.de
Dominga	Rogolino	University of Parma	dominga.rogolino@unipr.it
Ekaterina	Ryabchevskaya	Lomonosov Moscow State University	eryabchevskaya@gmail.com
Nicolò	Santi	Cardiff University	SantiN@cardiff.ac.uk
Sante	Scognamiglio	Università di Cagliari	santescognamiglio1@gmail.com
Saumya	Singh	Sam Higginbottom University of Agriculture, Technology & Sciences	singh.saumya0406@gmail.com
Irina	Stefanelli	Università di Napoli Federico II	irina.stefanelli@unina.it
Oriana	Tabarrini	Università di Perugia	oriana.tabarrini@unipg.it
Tyng Hwey	Tan	University of Aberdeen	t.tan.19@abdn.ac.uk
Joy E.	Thames	University of Maryland	Jthames1@umbc.edu
Charles	Waters	University of Maryland	Charlew1@umbc.edu
Dominik	Werz	University of Hambur	dominik.werz@chemie.uni-hamburg.de
Low Zhao	Xuan	Sunway University	19086693@imail.sunway.edu.my