



2nd IAAASS

Innovative Approaches for Identification of Antiviral Agents Summer School

September 28th – October 3rd 2014,
Santa Margherita di Pula, Sardinia, Italy

Program & Abstract book

Summer school organizing committee

**Enzo Tramontano
Elias Maccioni
Cristina Parolin
Stuart Le Grice**



REGIONE AUTONOMA DE SARDIGNA
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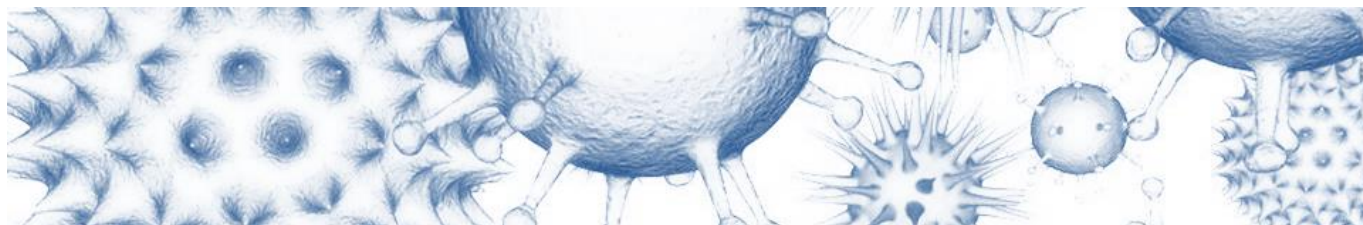


EUROPEAN SOCIETY
for VIROLOGY



**Società
Italiana di
Virologia**

International Antiviral Symposium Foundation



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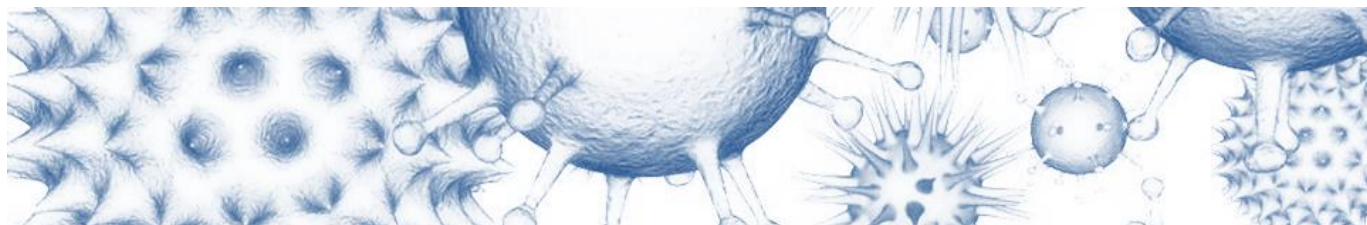
Following the success of the first edition, we have the pleasure to welcome you to the second “Innovative approaches for the identification of antiviral agents” summer school, with the patronage of Regione Autonoma della Sardegna, University of Cagliari, European Society for Virology, Federation of European Microbiological Societies, Italian Society for Virology and International Antiviral Symposium Foundation.

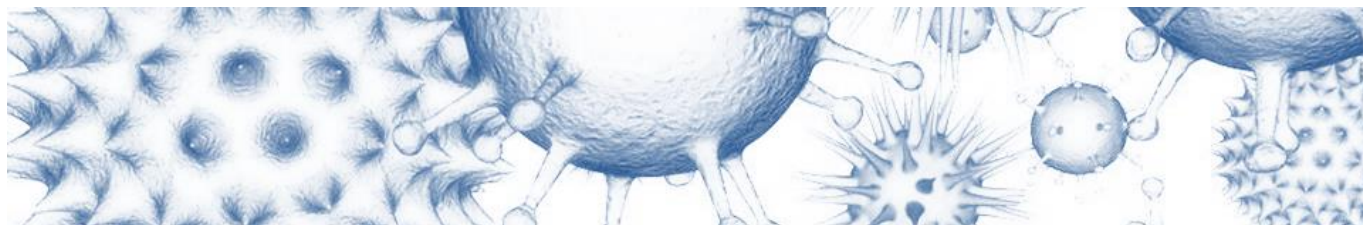
Over the recent years, drug discovery via high throughput screening (HTS) has moved beyond the boundaries of pharmaceutical companies and been successfully integrated into research programs of many academic institutes. These efforts have taken advantage of the availability of diverse libraries of small 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 to the Hotel Flamingo, Santa Margherita di Pula, located on the south tip of Sardinia, and looks forward to sharing with you their experience on current and future strategies for identifying novel antiviral agents targeted to clinically-significant diseases.

The summer school organizing committee





The organizing committee

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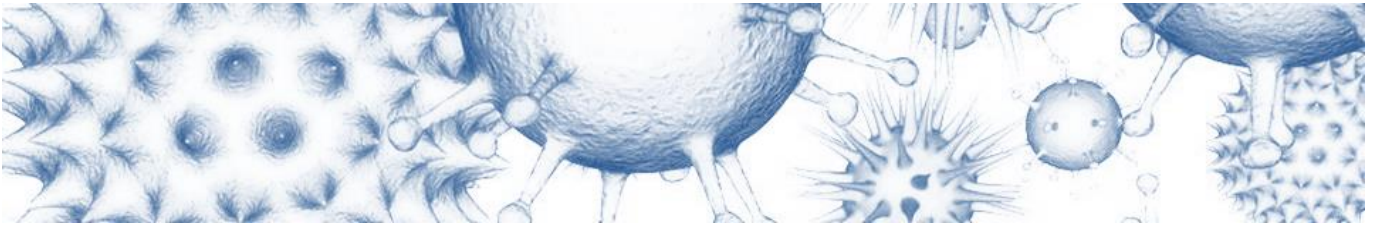
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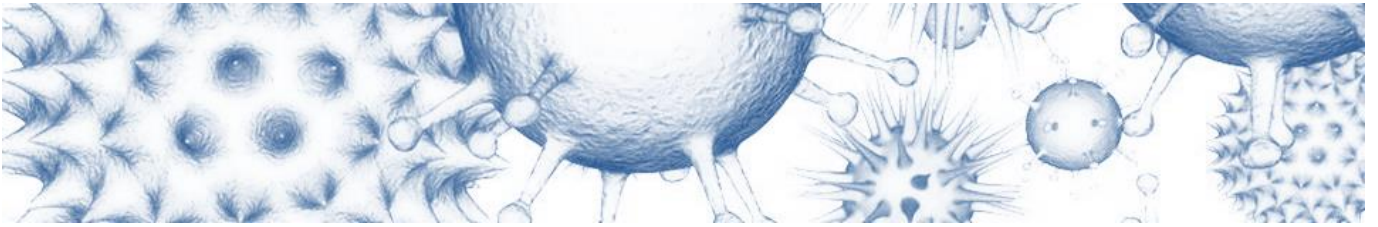
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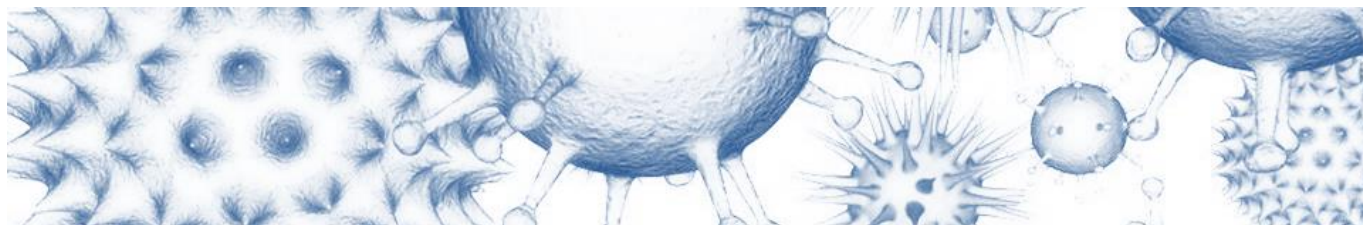
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IAAASS program



IAAASS program

2014.09.28

6.00 pm Registration

7.00 pm

Inaugural Lecture

Enzo Tramontano *Summer School welcoming remarks*

Giorgio Palù *President of the European Society for Virology welcoming remarks*

Carlo Federico Perno *Introduction to Inaugural Lecture*

Jan Balzarini *Carbohydrate binding agents: novel therapeutic concept as selective antiviral compounds.*

8.30 pm Dinner



2014.09.29

9.00 am

Plenary lectures

Chairman: Cristina Parolin

Stephan Becker	<i>Identifying targets for antiloviral interventions</i>
Maurizio Botta	<i>Design, synthesis and biological evaluation of human DDX3 inhibitors with multiple antiviral activities</i>

11.00-11.15 am	Coffee Break
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11.15 am

Chairman: Stuart Le Grice

Eric Freed	<i>Development of potent and broadly active HIV-1 maturation inhibitors</i>
Giorgio Palù	<i>Different approaches to develop effective antiviral strategies</i>

1.00 pm	Lunch
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3.30-5.00 pm	Discussion groups
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5.00-6.00 pm	Coffee break – Poster session I
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6.00-7.15 pm

Student talks

Chairman: Elias Maccioni

Florencia Linero	<i>Isolation and characterization of Nanobodies against Junin virus as new and improved treatment option for Argentine hemorrhagic fever</i>
Serena Massari	<i>Targeting Influenza A Virus RNA-dependent-RNA-polymerase: development of PA/PB1 interaction inhibitors</i>
Finny Varghese	<i>Novel antivirals affecting Alphaviruses, including the re-emerging Chikungunya virus</i>
Marta Cadeddu	<i>Identification and characterization of HERV-K (HML-10) in GRCh37/hg19 assembly and their possible association with the human diseases</i>
Pouya Hassandavish	<i>Mechanism study of baicalein activity against dengue virus replication</i>
8.00 pm	Dinner



2014.09.30

9.00 am

Plenary lectures

Chairman: Giorgio Palù

Le Grice Stuart	<i>Therapeutic Targeting of the Retroviral RNA Genome</i>
Mátyus Péter	<i>Through hardships to the drug candidate (Per aspera ad astra) – old and new concepts in HIV drug discovery</i>

11.00-11.15 am Coffee Break

11.15 am

Chairman: Eric Freed

Thomas Mertens	<i>Mechanisms, clinical problems and management of resistance against herpesvirus infections</i>
Birgitta Wöhr	<i>Inhibition of Foamy Virus RNase H by HIV-1 RNase H inhibitors development of a model system</i>

1.00 pm Lunch

3.30-5.00 pm **Discussion groups**

5.00-6.00 pm Coffee break – **Poster session II**

6.00-7.15 pm

Student talks

Chairman: Maurizio Botta

St. Patrick Reid	<i>The lipid kinase sphingosine kinase 2 is an essential host factor recruited by Chikungunya virus during infection</i>
Agharbaoui Fatima E	<i>From natural products to HIV-1 IN/LEDGF interaction inhibitors: computational and synthetic approaches</i>
Michele Celestino	<i>Unrevealing the molecular mechanisms accounting for the FIV envelope glycoprotein ability to overcome feline tetherin restriction</i>
Valeria Cannas	<i>Identification of VP35 dsRNA binding pocket useful residues for development of small-molecules inhibitors against Ebola virus</i>
Łukasz Świątek	<i>Antiviral activity of leaves and cones of <i>Alnus sieboldiana</i> Matsum against HHV-1</i>



8.00 pm

Dinner

2014.10.01

9.00 am

Plenary lectures

Chairman: Jan Balzarini

Reuben Harris *The Biological and Pathological Importance of Enzyme-Catalyzed DNA Cytosine Deamination*

Federico Gago *Structure-based computational strategies in the design of antiviral drugs*

11.00-11.15 am Coffee Break

11.15 am

Chairman: Thomas Mertens

Kvaratskhelia Mamuka *Multimerization selective inhibitors of HIV-1 integrase*

Enzo Tramontano *Are human endogenous retroviruses possible drug targets?*

1.00 pm Lunch

Free afternoon

8.00 pm Dinner



2014.10.02

9.00 am

Plenary lectures

Chairman: Stephan Becker

Ben Berkhout *New anti-HIV approaches: peptide inhibitors, RNAi gene therapy and the humanized mouse model for drug testing*

Maccioni Elias *Drug design: principles and considerations*

11.00-11.15 am Coffee Break

11.15 am

Round table on “Working in a company after the PhD”

Chairman: Stuart Le Grice

Participant: Franco Lori and Vincenzo Summa

1.00 pm Lunch

3.00-4.30 pm **Discussion groups**

4.30-5.00 pm Coffee Break

5.00-7.00 pm

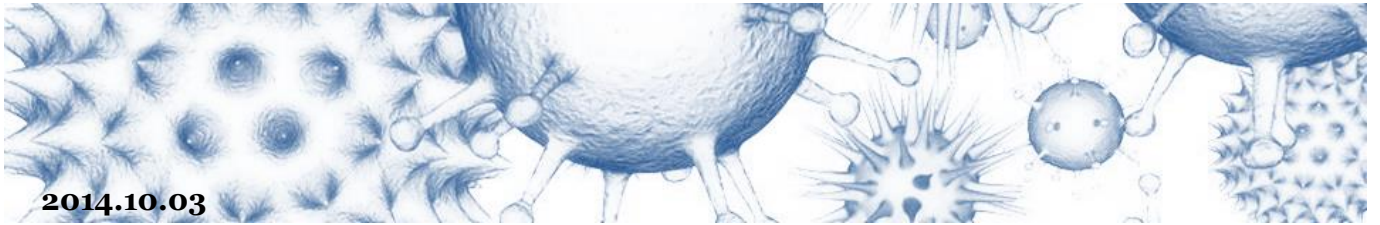
Plenary lectures

Chairman: Reuben Harris

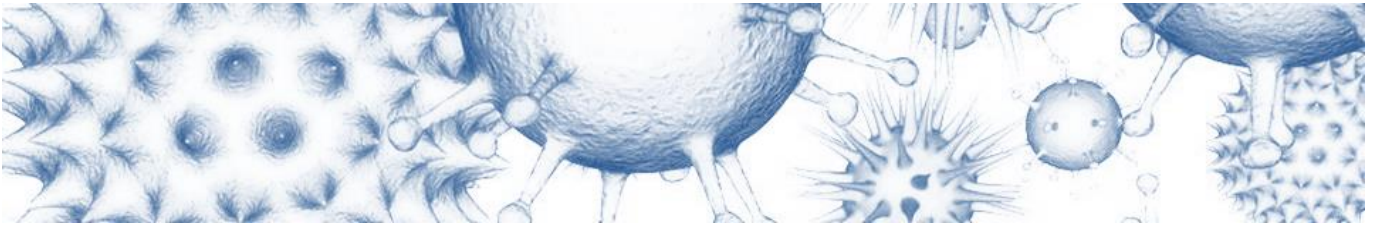
Roberto Di Santo *Inhibiting the HIV Integration Process: Past, Present, and the Future*

Camarasa María José *gp120 as a target of the design and discovery of HIV-entry/fusion inhibitors*

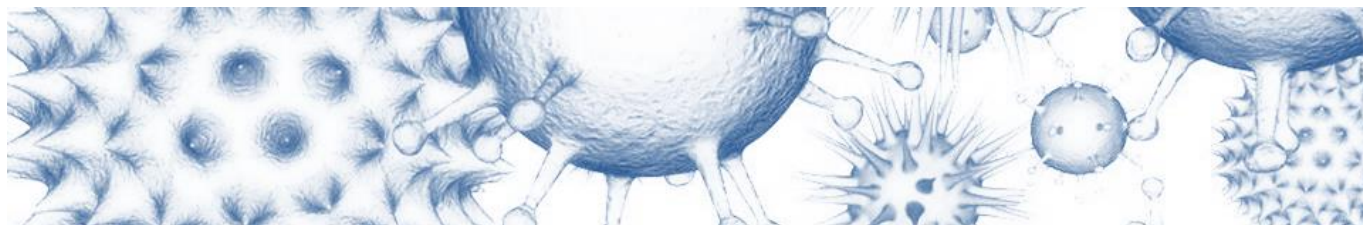
8.00 pm Social Dinner



Breakfast and leaving



Plenary lectures abstracts

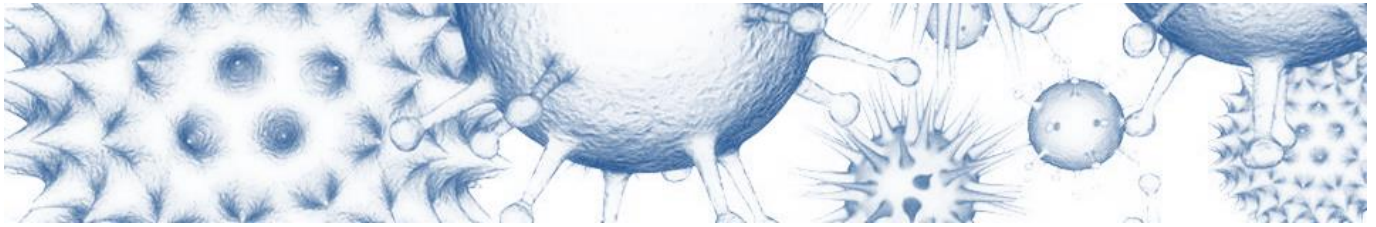


Carbohydrate-binding agents: novel therapeutic concept as selective antiviral compounds

Jan Balzarini

Rega Institute for Medical Research, KU Leuven, B-3000 Leuven, Belgium

Carbohydrate-binding agents (CBAs) can be isolated from a variety of species. A number of them, in particular those CBAs that show specific recognition for mannose (Man) display a remarkable activity against enveloped viruses (i.e. HIV, HCV) in cell culture. Also the non-peptidic α -1,2-Man-specific antibiotic Pradimicin markedly suppresses viral infection. Most CBAs function as oligomers. CBAs qualify as potential anti-HIV microbicide drugs because they inhibit infection of cells by cell-free virus, efficiently prevent virus transmission from virus-infected cells to uninfected T-lymphocytes, block HIV capture by DC-SIGN-expressing cells and compromise transmission of DC-SIGN-captured virus to CD4⁺ T-lymphocytes. Their most likely mechanism of antiviral action is the interruption of virus entry (i.e. fusion). CBAs presumably act by direct binding to the N-glycans on the HIV-1 gp120 envelope. They may cross-link several glycans during virus/cell interaction and/or freeze the conformation of gp120 preventing further interaction with the coreceptor. CBA pressure in HIV-infected cell cultures forces the virus to delete N-glycans on gp120 to escape CBA inhibition. Several CBAs have a high genetic barrier. CBAs are the prototypes of conceptionally novel chemotherapeutics with a unique mechanism of antiviral action, drug resistance profile and an intrinsic capacity to trigger a specific immune response against HIV strains after glycan deletions on their envelope occur in an attempt to escape CBA drug pressure.

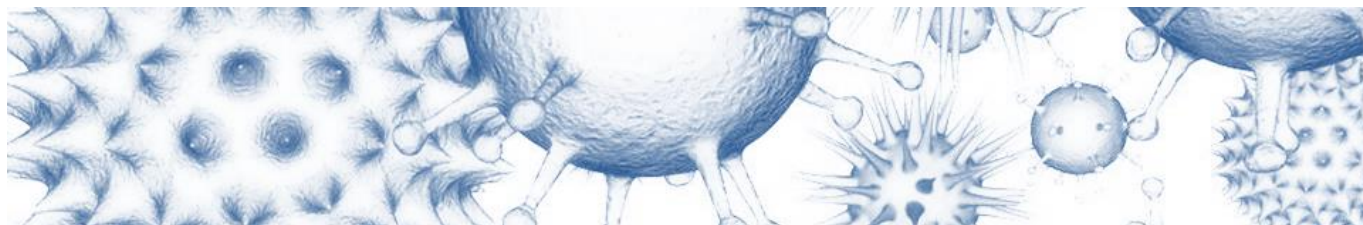


Identifying targets for antifiloviral interventions

Stephan Becker

Institut für Virologie, Philipps-University Marburg, Germany

Formation of Marburg virus particles inside infected cells is a modular process, which is highly regulated in time and space and needs the interaction of viral proteins and RNA with a plethora of cellular proteins. First, viral genomic RNA is encapsidated by five viral proteins in virus-induced inclusions in the perinuclear region giving rise to the formation of viral nucleocapsids (assembly step 1). Then, association of nucleocapsids with the matrix protein VP40 takes place in the cell periphery (assembly step 2) and finally, acquisition of the viral lipid envelope and the surface glycoprotein is accomplished via budding through the plasma membrane in filopodia, specialized actin-containing cellular protrusions (assembly step 3). We have analyzed the transport of nucleocapsids between the assembly sites and detected that actin polymerization is essential for their migration. To determine whether the actin-dependent transport of nucleocapsids represents a target for antiviral interventions we have tested a number of inhibitors that influenced cellular actin-dependent transport. We found that intracellular transport of Marburg and Ebola virus can be blocked efficiently by chemicals that inhibit actin polymerization.



Design, synthesis and biological evaluation of human DDX3 inhibitors with multiple antiviral activity.

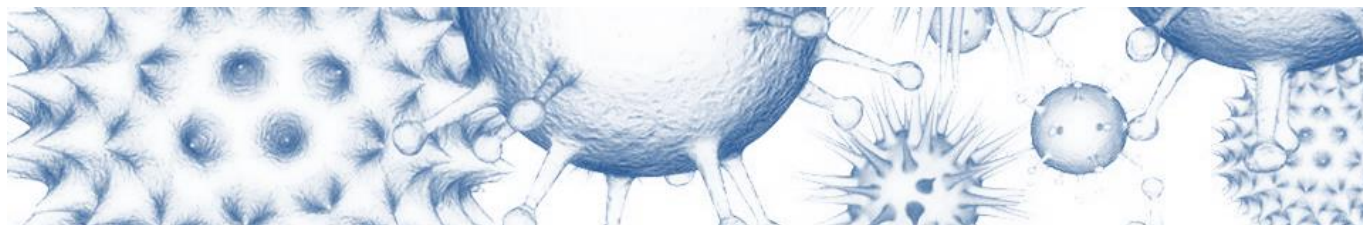
Maurizio Botta

Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Siena, Italy

Understanding the complex network of host-virus interactions can provide new avenues for the treatment of viral infections, in that essential cellular cofactors for viral replication represent attractive novel targets for antiviral therapy. Targeting cellular cofactors could have the advantage of limiting the occurrence of drug resistance, as cellular proteins are less prone to mutate than viral proteins. Recently, several independent studies have revealed that the cellular ATPase/RNA helicase X-linked DEAD-box polypeptide 3 (DDX3) is an essential host factor for some of the most clinically and economically challenging human pathogens: Human Immunodeficiency Virus, (HIV-1 Retroviridae), Hepatitis C Virus, Japanese Encephalitis Virus, Dengue Virus and West Nile Virus (HCV, JEV, DENV, WNV, Flaviviridae). DEAD-box helicases are involved in a large variety of cellular processes involving RNA, such as splicing, mRNA export, transcriptional and translational regulation and ribosome biogenesis. (1) Our research group is working in targeting both the ATPase and RNA binding regions of DDX3. (2)(3) In this regard the identification of the first small molecule, specifically designed to interfere with the RNA binding of hDDX3 has accomplished by us (4, 5). Subsequently we used molecular modeling methods to guide the synthesis of new derivatives in order to find new compounds with increased activity and improved pharmacokinetics proprieties. As a result, most of the new compounds were able to inhibit DDX3 helicase activity at micromolar/submicromolar concentration. Remarkably, some compounds showed anti-HCV and anti-HIV activity in cells, as well as a good inhibitory activity against JEV, DENV and WNV infections. No cytotoxicity was found for the studied compounds up to 20 μ M concentration. Furthermore, our compounds proved to be metabolically stable in a range of 80-90 % in presence of human microsomes.

Literature

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3. Maga G., Falchi F., Radi M., Botta L., Casaluce G., Bernardini M., Iranrjad H., Manetti F., Garbelli A., Zanolli S., Esté J., Gonzalez E., Zucca E., Paolucci S., Baldanti F., De Rijck J., Debyser Z., Botta M., 2011, *Chem. Med. Chem*, 6:1371
4. Garbelli A., Radi M., Falchi F., Beerman S., Zanolli S., Manetti F., Dietrich U., Botta M., Maga G., 2011, *Curr. Med. Chem*, 18:2094
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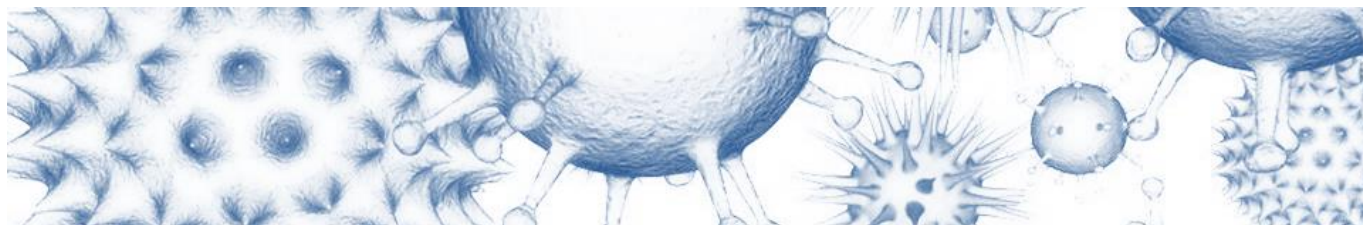


Development of highly potent and broadly active HIV-1 maturation inhibitors

Eric Freed

Head, Virus-Cell Interaction Section
Deputy Director, HIV Drug Resistance Program Center for Cancer Research
National Cancer Institute Frederick MD, USA

The Gag polyprotein precursor is responsible for the formation and release of virus particles from infected cells. Following release, particle maturation is triggered by the cleavage of the Gag polyprotein precursor by the viral protease (PR). Maturation is essential for particle infectivity. We previously reported on the first-in-class maturation inhibitor, bevirimat, which acts by blocking a late step in Gag processing. We also examined the activity of a structurally distinct maturation inhibitor reported by Pfizer (PF-46396) and selected for resistant mutants that displayed a striking degree of compound dependence in their assembly and replication. These compound-dependent mutants contained amino acid substitutions in a region of Gag known as the 'major homology region' (MHR), a highly conserved sequence that is required for virus assembly. The identification and characterization of maturation inhibitor-resistant mutants have provided insights into the target and mechanism of action of this class of inhibitors. Our recent work has led to the development of inhibitors that are highly potent and broadly active relative to the parent compound, bevirimat. These findings highlight the potential for the Gag processing and maturation step of the HIV-1 replication cycle as a target for novel antiretrovirals.

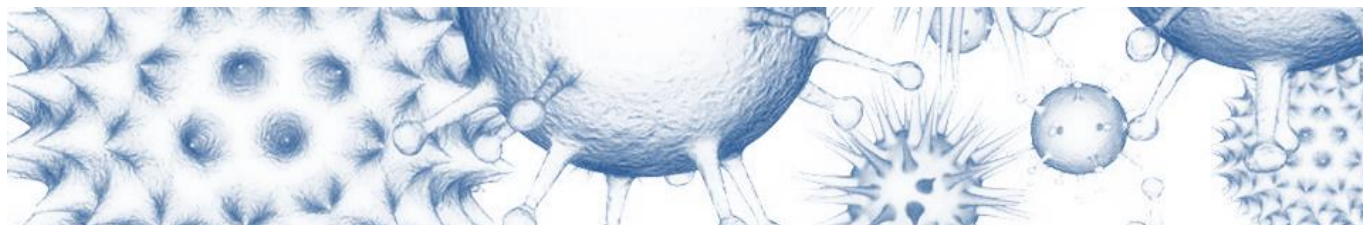


Different approaches to develop effective antiviral strategies

Giorgio Palù

Department of Molecular Medicine, Director, University of Padova, Italy

The presentation will describe different approaches pursued in the search of newer antiviral agents able to recognize specific viral targets, unrelated to the host cell physiology. The aim is selectivity, the core of antiviral research, not an easy task to satisfy by both academic labs and industry. Among the followed strategies is the identification of unique interactions, involving drugs, nucleic acids and proteins, which are driven by the stereospecificity of atomic contacts in the supramolecular configuration of the complex. Examples will be given of the i) use of antisense and ribozyme molecules as well as of siRNA and intrabodies to curb HIV replication; ii) deployment of drugs able to recognize the secondary structure of G quadruplex and to affect HIV and HSV gene expression; iii) identification of peptides and small molecules able to disrupt protein-protein interactions essential for replication of HSV, HCMV, influenzavirus, the targets being mainly the tridimensional structure of enzymatic proteins. Insights will also be given on how the inhibitory small molecules are selected either by high throughput screening of chemical libraries or by *in silico* molecular dynamics programmes. Examples of lead identification are described that are then followed by optimization of the chromophore using classical SARS and scaffold hopping. The search of compounds with broad antiviral activity, based on the inhibition of cellular as opposed to viral pathways, is described for Ebolavirus as a representative model of etiologic agents responsible for acute, fatal infections for which an effective therapy is still on demand.



Therapeutic Targeting of Viral RNAs: High-Hanging Fruit Only Needs a Longer Ladder

Stuart F.J. Le Grice

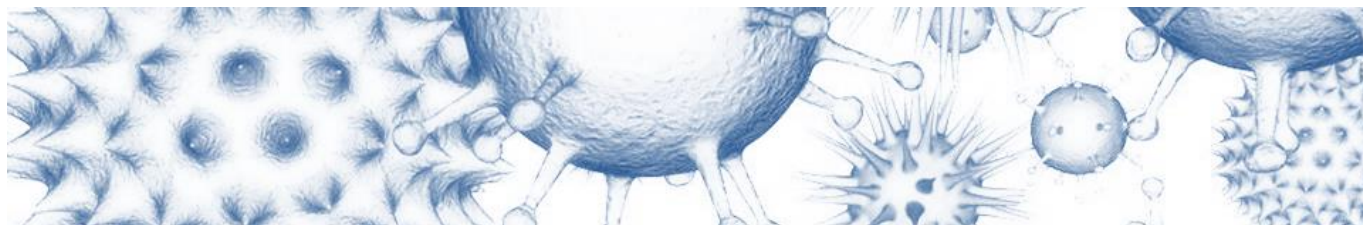
Reverse Transcriptase Biochemistry Section, HIV Drug Resistance Program, National Cancer Institute – Frederick, Frederick MD, USA.

Small molecules targeting the enzymes responsible for human immunodeficiency virus (HIV) polyprotein maturation (protease), DNA synthesis (reverse transcriptase) and the subsequent insertion of ribonucleotide-free double-stranded DNA into the host chromosome (integrase) have for several years been the central components of combination antiretroviral therapy. For infected individuals harboring drug-susceptible virus, this approach has afforded complete or near-complete viral suppression. However, in the absence of a curative strategy, the predictable emergence of drug-resistant variants requires continued development of improved antiviral strategies, inherent to which is the need to identify novel targets. Cis-acting regulatory elements of the HIV-1 RNA genome that regulate its transcription (the transactivation response element, TAR), translation (the ribosomal frameshift signal), nucleocytoplasmic transport (the Rev response element or RRE), dimerization (the dimer linkage sequence or DLS), packaging (the Ψ element) and reverse transcription of the (+) strand RNA genome (the primer binding site, or PBS) should now be considered as alternative targets for small molecule, peptide- and oligonucleotide-based therapeutics, as well as combinations thereof. The first part of this talk will summarize how high-resolution 3D structural information is being used to develop small molecule and peptide-based therapeutics that target critical cis-acting RNA motifs of the HIV-1 genome and consequently may be less prone to resistance-conferring mutations. Subsequently, advances in the development of novel high-throughput small molecule microarrays (SMMs) and RNA motifs that have been successfully targeted by this approach will be presented. An extension of the (SMM) approach to target other viral RNAs, or virus-specified RNAs, will be presented. Finally, where target specificity, endosomal release, cellular penetration and toxicity have been the primary obstacle to successful “macromolecule therapeutics”, methodological advances will be reviewed.

This work is supported by the Intramural Research Program of the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, USA

Literature

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2. Sztuba-Solinska, J., Shenoy, S.R., Gareiss, P., Krumpe, L.R.H., Le Grice, S.F.J., O’Keefe, B.R. and Schneekloth, J.S. (2014). Identification of biologically-active, HIV TAR RNA-binding small molecules using small molecule microarrays. *J. Am. Chem. Soc.* 136, 8402-8410.
3. Sai Pradeep, V., Gallo, S.M. and Disney, M.D. (2014). Sequence-based design of small molecules that target precursor microRNAs. *Nat. Chem. Biol.* 10, 291-297.



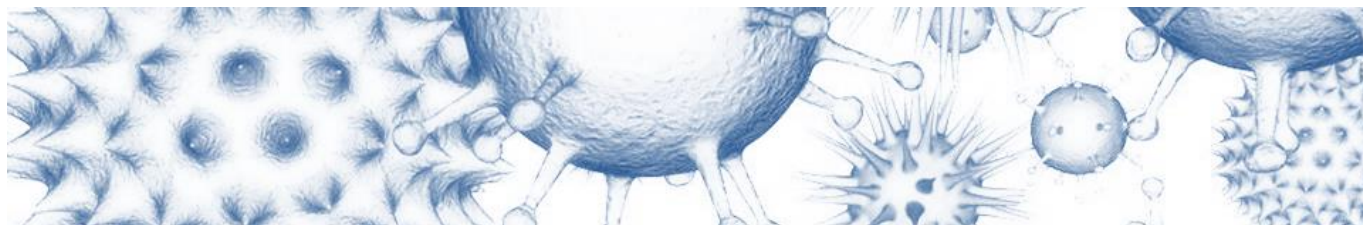
Through hardships to the drug candidate (*Per aspera ad astra*) – Old and new concepts in HIV drug discovery

Péter Mátyus

Department of Organic Chemistry, and Drug Discovery and Safety Centre, Semmelweis University, Budapest, Hungary

Although remarkable new results and tools have been achieved and developed in biomedical sciences as well as pharma R&D spending and investments in novel discovery technologies, such as combinatorial chemistry and high-throughput screening (HTS) have been enormously increasing over the last two decades, in fact, they could not be translated into high productivity as measured by the number of original drugs introduced in the therapy. Medicinal chemistry has always been a key player in drug discovery. In its traditional role, it provided drug candidates by optimizing a targeted biological activity. Failures in clinical development due to unfavorable ADMET properties have led to new multidimensional lead optimization, more recently, based on 'rational' (target structure- and/or metrics-driven) strategies, to improve compounds' quality by shaping lead (candidates) also for desired pharmacokinetic and selectivity profile. Nonetheless, all these approaches, due to their own limitations, have performed only with a limited success rate in providing the winning drug candidate in a predictable, cost-effective way. It is therefore thought that the innovation process itself needs new inputs and careful reconsideration of the full pathway to bridge the productivity gap. To improve performance of R&D, we believe that much more attention should be paid to target selection and validation, which also relates to compounds' selectivity vs (designed) dual/poly-pharmacology, whereas creative utilizations of existing drug pools also deserve consideration for clinical drug discovery. From organizational point of view, establishment of new types of co-operations among clinicians, medicinal chemists, bioinformatics scientists, molecular biologists and pharmacologists is also essential.

The examples we have taken illustrate traditional and new roles of medicinal chemistry in the discovery of anti HIV drug candidates and drugs acting on new targets or multiple targets or used in combination.



Mechanisms, clinical problems and management of resistance against herpes virus infections

Thomas Mertens

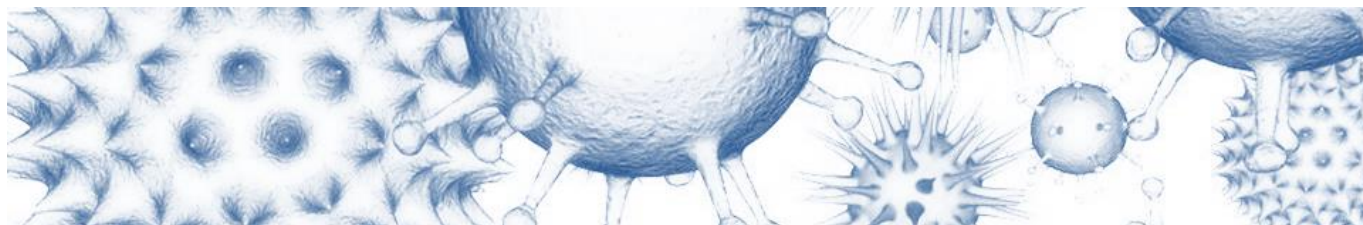
Institute of Virology at the Ulm University Hospital, Ulm, Germany

Although the first antiviral substances have been developed against herpes viruses, today only a restricted number of compounds targeting the viral polymerase have been licensed for the treatment of herpes virus infections. New compounds acting on different targets are urgently needed since e.g. concerning human cytomegalovirus (HCMV) the number of immunosuppressed patients needing therapy has dramatically increased and there is no drug available for the safe treatment of primary infections in pregnant women.

The selection of resistant HCMV is a growing problem in clinical settings and virologists have to monitor patients under therapy accordingly. There exist a number of open questions concerning the kinetic of selection of resistances and multiple resistances. Namely, potential interferences between sensitive and resistant viral subpopulations, the role of viral fitness and compensatory mutations. Multi resistance may be diagnosed if mixed viral populations occur carrying different resistance conferring mutations but also if single viral genomes carry multiple mutations.

Viral resistance can be determined by phenotypic or genotypic testing. Genotypic testing is faster and provides other advantages, but every mutation has to be characterized phenotypically after marker transfer to allow interpretation of genotypic results. Furthermore, different resistance conferring mutations lead to different degrees of resistance. So finally the overall clinical resistance of a virus population is depending on the amount of the resistant virus subpopulation, the type of mutation(s) and the fitness of mutated viruses. Virologists have to provide methods and data in the laboratory which help to make therapeutic decisions on a rational basis.

Some new compounds have reached clinical studies and others are in “the pipeline” but new targets for antiviral intervention have to be identified. Flanking the development of antiviral therapy the role of immunotherapy (adaptive and innate) has to be determined and methods for application have to be developed.

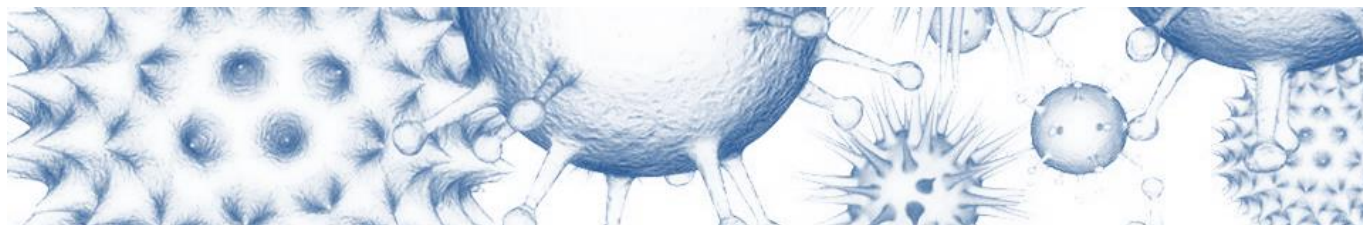


Inhibition of Foamy Virus RNase H by HIV-1 RNase H inhibitors - development of a model system.

Birgitta Wöhr

Department of Biopolymers, Universität Bayreuth, Germany

The ribonuclease H (RNase H) plays an essential role in the replication of retroviruses. Therefore, it is a promising target for drug development against human immunodeficiency virus type 1 (HIV-1). We have previously solved the structure of the prototype foamy virus (PFV) RNase H and demonstrated that HIV-1 and PFV RNase H exhibit a high degree of structural similarity. Therefore, we examined whether PFV RNase H can serve as an HIV-1 RNase H model system for inhibitor interaction studies. Identification of HIV-1 RNase H inhibitors has been hindered by the open morphology of its active site, the limited number of available RNase H/inhibitor co-crystals and the fact that due to the high concentrations of Mg ions needed for protein stability, HIV-1 RNase H is not suitable for NMR inhibitor studies. Five HIV-1 RNase H inhibitors were tested in vitro with purified full length PFV protease- reverse transcriptase (PR-RT) and inhibited the PFV RNase H at low micromolar concentrations, similar to those of HIV-1 RNase H, suggesting pocket similarity of the two RNase H domains. We performed NMR titrations experiments with the purified PFV RNase H domain and the inhibitor RDS1643 (6-[1-(4-fluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic acid ethyl ester) to determine its binding site. Based on these results and previous data, in silico docking analyses showed a putative RDS1643 binding region that reaches into the PFV RNase H active site. Structural overlays were performed with HIV-1 and PFV RNase H to propose the RDS1643 binding site in HIV-1 RNase H. Our results suggest that this approach can be used to establish PFV RNase H as a model system for HIV-1 RNase H in order to identify putative inhibitor binding sites in HIV-1 RNase H.



The Biological and Pathological Importance of Enzyme-Catalyzed DNA Cytosine Deamination

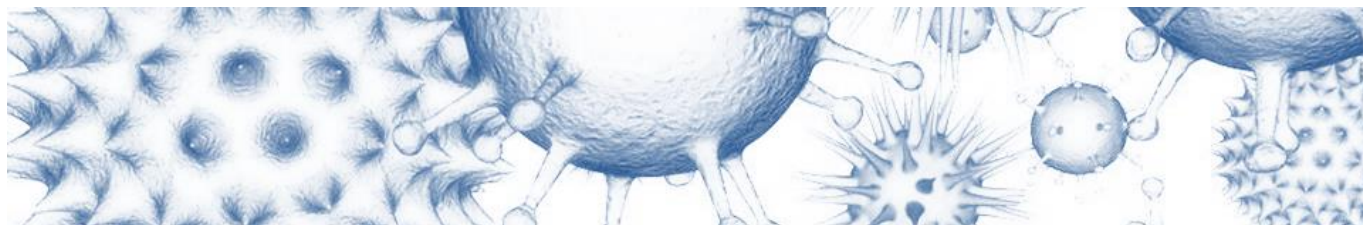
Reuben Harris

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The human APOBEC family of polynucleotide cytosine deaminases is comprised of nine active enzymes: APOBEC1, AID, APOBEC3A/B/C/D/F/G/H. The namesake, APOBEC1 is a bona fide RNA editing enzyme that is guided by an editing complex to physiological target mRNA species such as the APOB message. APOBEC1 also has robust DNA cytosine editing activity and has been implicated recently in esophageal adenocarcinomas. AID activity appears specific to DNA cytosines, and this enzyme is essential for antibody gene diversification by somatic hypermutation and class switch recombination. AID activity a likely contributor to mutagenesis and driver events in B cell cancers. The seven APOBEC3 enzymes have strong biochemical preferences for single-stranded DNA substrates and have been broadly implicated in providing innate immunity to parasitic DNA elements including naked DNA, viruses, and transposons. At least one APOBEC3 subfamily member, APOBEC3B, is a source of mutation in many different cancer types (1-3). This talk will review recent progress in this area and discuss implications for mutation-dependent cancer outcomes including recurrence, metastasis, and the development of therapy resistant tumors (4).

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Structure-Based Computational Strategies In The Design Of Antiviral Drugs

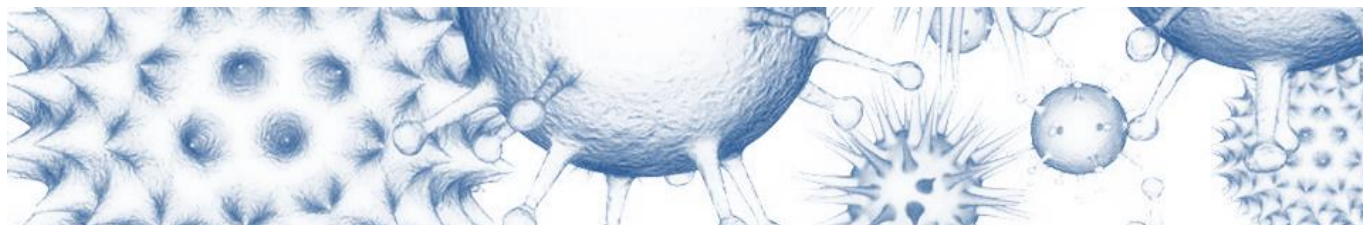
Federico Gago

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X-ray crystallography, NMR spectroscopy, and electron cryomicroscopy stand out as powerful structural biology tools that enable us to obtain atomic detail about biomolecules that can be potentially targeted by drugs. This knowledge is essential if virtual screening or structure-based ligand design methods are going to be used in the drug discovery effort. However, not always is the macromolecule of interest amenable to this type of experiments or, as is often the case, the conformation found experimentally cannot be used directly for docking studies because of significant changes between apo and ligand-bound forms. Furthermore, the desired insight into the binding mechanism cannot be gained sometimes because the structures of the ligand-receptor complexes that are available represent the end-point of the binding process and therefore retain little or no information about the intermediate stages that led to their creation. To circumvent these limitations and gain additional insight into the motions and rearrangements involved some theoretical methods, including normal mode analysis and molecular dynamics simulations, can be of help as they allow an improved sampling of conformational space.

On the other hand, a variety of tools have been developed to (1) identify and characterize binding pockets within proteins, (2) dock within these sites and score putative ligands (and molecular fragments) from selected chemical libraries for ranking purposes, (3) carry out ligand-based similarity searches to perform "scaffold hopping", and (4) better understand how chemical modifications in a series of ligands bring about changes in their binding affinities (structure-activity relationships).

My presentation will provide an overview of these methodologies as well as their application to several viral targets.

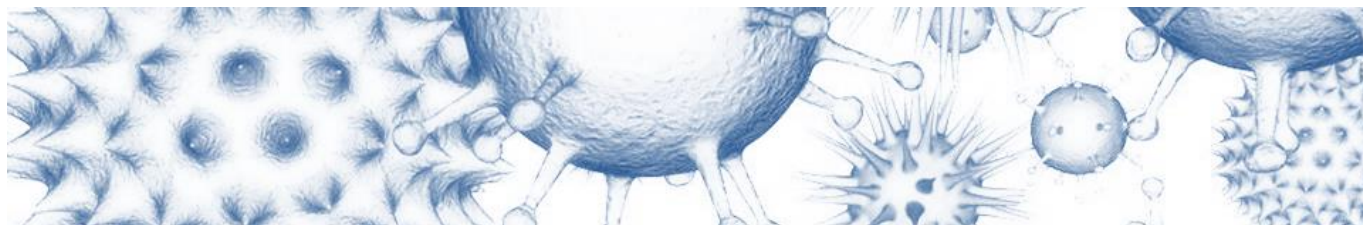


Allosteric inhibitors inducing aberrant multimerization of HIV-1 integrase

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Emergence of HIV-1 phenotypes resistant to current therapies is a major clinical problem. Therefore, there is a continued need for developing inhibitors with new mechanisms of action. One such mechanism is to modulate functionally essential multimerization of a key viral protein HIV-1 integrase. We have designed pyridine-based small molecules that contain a rotatable single bond to allow optimal bridging between interacting integrase subunits. As a result pyridine-based compounds stabilized interacting IN subunits and promoted aberrant, higher order integrase multimerization. The most potent compound, KF116, inhibited HIV-1 replication with EC₅₀ of 24 nM by interfering with proper maturation of HIV-1 particles, whereas KF116 at therapeutically relevant (submicromolar) concentrations had no detectable effects on LEDGF/p75 mediated HIV-1 integration. Our findings highlight HIV-1 integrase multimerization as a plausible therapeutic target during the late phase of HIV-1 replication and offer a path for designing improved inhibitors for potential clinical use.

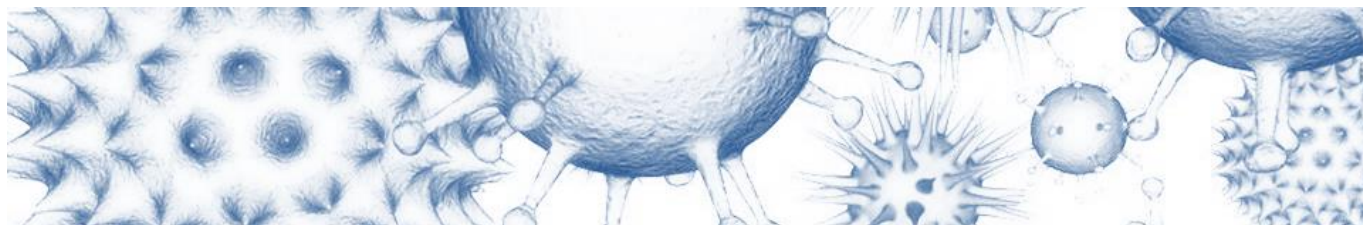


Are human endogenous retroviruses possible drug targets?

Enzo Tramontano

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Recent estimates indicate that about 8% of the human genome consists of retroelements including Human Endogenous Retroviruses (HERVs) that represent the inheritance of ancient (up to 100 million of year ago) germ-line cell infections by exogenous retroviruses and the subsequent transmission of the proviral integrated elements to the descendants. While no replication-competent HERV sequence is recognizable in the human genome, some HERVs retain one or several intact retroviral genes and may express protein products that could have a physiological/pathological role. In fact, some HERV proteins have been 'exapted' and have a physiological role in human placenta functioning, while in a number of autoimmune diseases such as systemic lupus erythematosus, insulin-dependent diabetes mellitus, multiple sclerosis, rheumatoid arthritis and others, high levels of HERVs expression has been observed. In addition, HERV over expression has been detected also in a number of cancers, however it is not clear if these HERV over expressions are due to a bystander effect, that could be eventually used as disease marker, or are, at least partially, disease determinants. Such investigations are hampered by the lack of accurate information of HERV copy number, position, integrity and expression potential. Hence, we analyzed the human genome assembly GRCh37/hg19 with RetroTector software and identified and characterized more than 3000 HERV proviral sequences. HERVs were classified in 40 unique clades (groups) which could be placed into class I (Gamma- and Epsilon-like), II (Beta-like) and III (Spuma-like). Noteworthy, the presence of a high number (around 40%) of mosaic forms, with heterogeneous sequence content, was observed. A finest characterization of the HERV sequences was achieved with the investigation of a broad panel of structural markers that contributed to confirm and extend the previously performed classification. Integration patterns analysis showed a tendency for proviruses from the same clade to occur together, within 100,000 bases, maybe due to local duplications. Representatives from some Gammaretroviral clades (HERVH and HERVE) integrated more frequently than expected by chance into the 5'-end of transcriptional units, mostly in antisense orientation.



New anti-HIV approaches: peptide inhibitors, RNAi gene therapy and the humanized mouse model for drug testing

Ben Berkhout

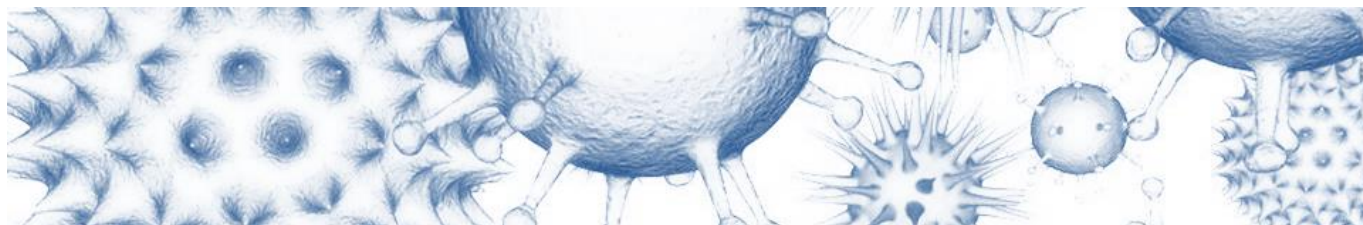
Department of Medical Microbiology Center for Infection and Immunity Amsterdam (CINIMA) The Netherlands

There are currently more than 25 potent antiviral drugs available to control HIV-1 replication and disease progression in infected individuals. Nevertheless, problems may arise due to the selection of drug-resistant virus variants, drug-related toxicity due to the required daily drug intake for many years, and the cost of these drugs may restrict their usage in some parts of the world. Thus, new antiviral approaches are needed, both new drug classes that target additional viral functions and new therapeutic approaches.

I will discuss the status of peptidic drugs that target the process of membrane fusion between virus and the target cell. More and more potent peptides were generated by the pharmaceutical industry, but their clinical use is restricted by the cost of production and the requirement for peptide injection. We tested the performance of 3 generations of anti-HIV peptides in cell culture models. We also probed the likelihood of selecting drug-resistant viruses, studied the underlying molecular mechanisms, which in fact provided novel insight about how to further improve this class of antivirals. Such peptides may form a major drug class for emerging viral pathogens such as SARS and MERS that all use similar fusion mechanisms.

A gene therapy that protects cells against HIV-1 infection is proposed as a future application because it holds the promise to present a single treatment with a long-lasting therapeutic effect. The development of a gene therapy will be discussed that is based on the mechanism of RNA interference (RNAi). The selection of potent RNAi-based inhibitors will be described, followed by a detailed analysis of viral escape routes and the design of an anti-escape RNAi cocktail. The idea is to modify human hematopoietic stem cells *ex vivo* with a lentiviral vector that encodes multiple RNAi-based inhibitors that target the HIV-1 RNA genome.

Testing of new drugs and the RNAi gene therapy requires new animal models as the SIV-macaque model is expensive, ethically constrained and not always an accurate mimic of the HIV-human context. I will describe a new humanized mouse model and its use for testing novel antiviral drugs, but also the efficacy and safety of the RNAi gene therapy. There is much recent discussion on the development of a sterilizing HIV-1 cure. The enormous obstacles along this route towards virus eradication from the infected individual will be discussed.

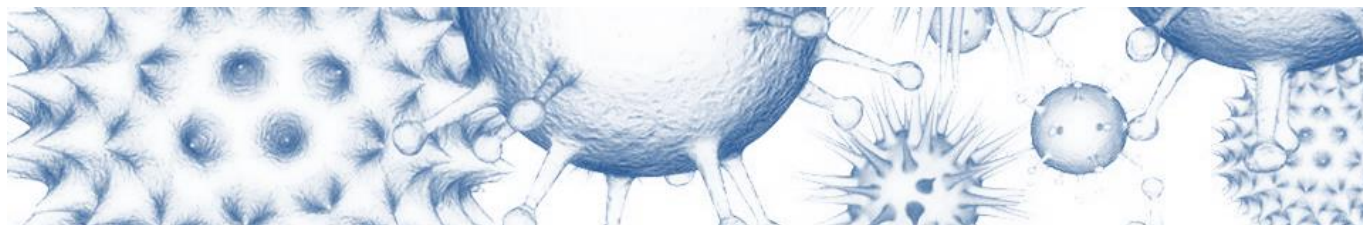


Drug design: principles and considerations

Elias Maccioni

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The discovery of new drugs is a long cost demanding process. In the past new drugs were mainly identified from traditional remedies and herbs. More recently chemical libraries of small molecules or natural compounds were screened in the hope of finding a desirable therapeutic effect. Modern drug discovery involves the rational design of hit molecules for specific biological targets and their optimisation to increase affinity, selectivity, potency, metabolic stability and bioavailability. All of these steps require the collaboration of a network of scientists with different multidisciplinary expertise. In this respect the role of medicinal chemist has undergone dramatic changes. Since Paul Ehrlich introduced the concept of chemotherapeutic agents, drug design does not rely only on chemistry anymore, but instead requires highly planned processes where knowledge about enzymes and receptors has suggested a dialogue between chemists and biologists. Thanks to the help of molecular biology and computational techniques medicinal chemists can now synthesise new drug molecules rationally designed for specific targets. By this approach the risk associated with the drug discovery program is highly reduced as the use of molecular modelling, in silico toxicology and ADME software is designed to further mitigate risk through eliminating analogues that may show unfavourable toxicity and/or bioavailability prior to compound synthesis.



Inhibiting the HIV Integration Process: Past, Present, and the Future

Roberto Di Santo

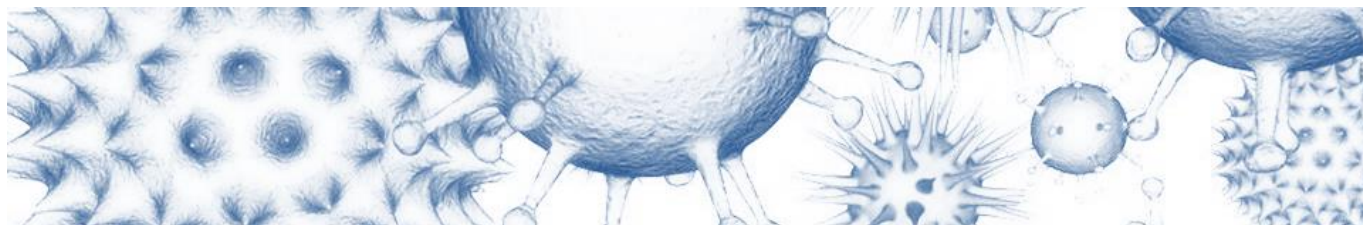
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Considerable success has been achieved in the treatment of HIV-1 infection, and many drugs are available targeting several distinct steps in the viral replication cycle. However, resistance to these compounds emerges readily, even in the context of combination therapy. Drug toxicity, adverse drug-drug interactions, and accompanying poor patient adherence can also lead to treatment failure. Finding new drugs, new drug targets and new therapeutic strategies may lead to redefining the goals of antiretroviral therapy, with an attempt to achieve the ultimate objective: the eradication of infection.

HIV integrase (IN) catalyzes the insertion into the genome of the infected human cell of viral DNA produced by the retrotranscription process. The discovery of raltegravir validated the existence of the IN, which is a new target in the field of anti-HIV drug research. The mechanism of catalysis of IN is depicted, and the characteristics of the inhibitors of the catalytic site of this viral enzyme, as well. The role played by the resistance is elucidated, as well as the possibility of bypassing this problem. New approaches to block the integration process are depicted as future perspectives, such as development of allosteric IN inhibitors, dual inhibitors targeting both IN and other enzymes, inhibitors of enzymes that activate IN, and activators of IN activity, as well as a gene therapy approach.

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Gp120 As A Target For The Design And Discovery Of HIV-Entry/Fusion Inhibitors

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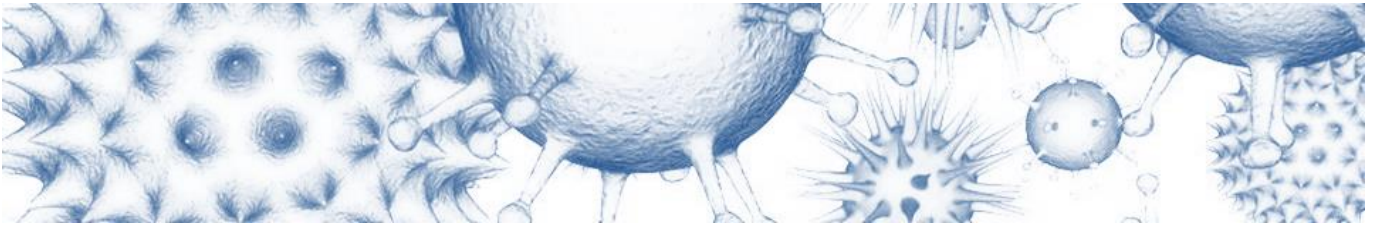
With around 40 million people infected with HIV alive nowadays, and a higher number expected in the future, HIV-infection is likely to be one of the most common chronic infectious disease in the 21 st century. There are several effective drugs, acting on different viral targets, that are used in cocktails in HAART therapy to treat HIV infections. This HIV treatment has proven successful and progression to AIDS has become increasingly rare in several parts of the world. However, the complexity of selecting and following a regimen, the potential side effects, the drug-resistance development, the lack of adherence from patients compromising the prevention of drug resistance, and the transmission of drug-resistant virus to the currently approved drugs, points out to the need of additional, more selective and less toxic, compounds acting at targets other than those employed by the current drugs and with a better pharmacological profiles.

Innovative "druggable" targets are being sought because of the eventual emergence of resistance or the inefficiency of current treatments with the common goal of discovering new compounds through the integration of some novel strategies, both in terms of macromolecular targets and/or mechanisms of inhibition. In this respect, small molecules able to block HIV fusion/entry process are particularly attractive candidates. Therefore, we focused on a novel and promising approach, so far unexploited, for the development of HIV replication inhibitors, namely, low-molecular-weight "lectin mimetics".

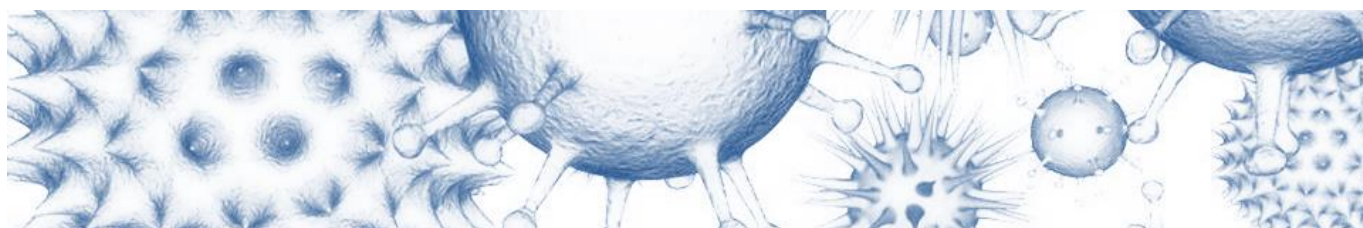
We designed molecules with the potential of preventing entry of the virus into the cell by interacting with the glycans of the gp120 viral protein. The feasibility of this approach has already been demonstrated for some natural lectins and for "artificial lectins". This is a novel therapeutic concept to fight against HIV infection that pursue a "direct" effect, by preventing HIV entry and transmission, and an "indirect" effect by forcing the virus to mutate and remove glycans from its envelope gp120 glycoprotein thus allowing the immune system to become triggered to suppress HIV more efficiently.

Acknowledgments

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Short talks abstracts



Isolation and characterization of Nanobodies against Junin virus as new and improved treatment option for Argentine hemorrhagic fever

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Argentine Hemorrhagic Fever (AHF) is an endemic disease specific of the central part of Argentina with a case-fatality rate, without treatment, ranging from 15% to 30%. Although the use of a live attenuated vaccine Candid#1, has markedly reduced the incidence of AHF in the population at risk, supportive therapy remains very important in the management of patients with AHF.

In this work we explored the development of a novel and improved therapeutic intervention against AHF, based on the generation and use of single domain antibodies (also known as Nanobodies or VHH). This technology combines unparalleled antigen-targeting specificities with ease of expression, purification and re-engineering opportunities.

The generation of the nanobodies directed against JUNV was performed by the immunization of an alpaca with purified UV-inactivated Candid#1 JUNV. After immunization, a cDNA library was generated and subsequently used to isolate phages that express JUNV-binding nanobodies at their surface by biopanning. The JUNV-binding capacity of these VHHs was evaluated by ELISA from the periplasmic extracts and the neutralizing activity of these binders was then evaluated functionally by a virus neutralization assay against JUNV Candid#1 strain. Results showed that 4 of the 70 candidates were able to neutralize successfully Candid#1 JUNV strain replication at very low concentrations. These findings constitute the first demonstration that the nanobody technology could be used to avoid JUNV replication. Afterwards testing of the virus-neutralizing activity against pathogenic strains will allow demonstrating the potential use of this technology treat and potentially preventing arenavirus hemorrhagic fever in human.

Targeting Influenza A Virus RNA-dependent-RNA-polymerase: development of PA/PB1 interaction inhibitors

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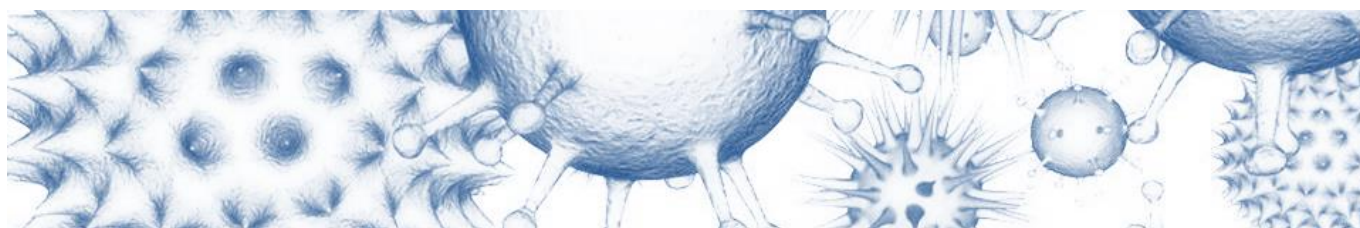
The currently available anti influenza (Flu) drugs, i.e. M2 blockers and neuraminidase inhibitors, are still inadequate to treat Flu virus, an important respiratory pathogen responsible for both yearly seasonal epidemics and more extensive global pandemics. Next generation antivirals with an innovative mechanism of action are urgently needed. In this context, the viral RNA-dependent RNA polymerase, a heterotrimer formed by the PB1, PB2, and PA subunits, and in particular its correct assembly, recently emerged as an attractive target.

Using the crystal structure of a truncated form of PA bound to a PB1-derived peptide, an in-silico screening of small molecule libraries recently led us to identify some promising hit compounds able to specifically interfere with the PA/PB1 interaction.¹ One of the most interesting hits has already undergone a first hit-to-lead optimization phase, leading to an increased PA/PB1 interaction inhibition and a more potent anti-Flu activity also encompassing clinical isolates and drug-resistant strains.² The preliminary SAR insights are guiding the design of further analogues. In parallel, a large series of derivatives was planned starting from a second hit. The design, synthesis, and biological evaluation of the two series of compounds will be the object of this presentation.

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Novel antivirals affecting Alphaviruses, including the re-emerging Chikungunya virus

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Chikungunya virus (CHIKV) is an *Alphavirus* and is a single-stranded positive sense RNA virus. It is a re-emerging arbovirus, endemic to Africa, South-east Asia and the Indian sub-continent and causes a persistent debilitating arthralgia along with fever, nausea, headache and a maculopapular rash. Over the last decade, the virus has re-emerged with several explosive epidemics and has also found its way to Europe, the Far East and even the Caribbean islands in the western hemisphere. Using a luciferase reporter based CHIKV replicon containing cell line; we have screened approximately 3000 small molecular weight compounds from three different compound libraries. Following initial validation, 25 compounds were chosen as primary hits for further confirmatory studies. Thereafter, secondary validation was performed with wild-type and reporter CHIKV infection based assays to narrow this list down to three promising compounds. A1, B1 and G1 inhibited CHIKV replication in a dose-dependent manner. They also have broad anti-viral activity against other alphaviruses – Semliki Forest virus (SFV) and Sindbis virus (SINV) and some are potent against Yellow Fever virus (YFV), a flavivirus. These compounds also showed reduced synthesis of genomic and antigenomic viral RNA in a Northern blot assay as well as downregulation of viral protein expression, visualized through a Western blot assay performed on cell lysates of compound-treated CHIKV infected cells. Time of addition studies were done to determine a putative mode of action for these compounds. While A1 and G1 most likely act on the replication phase of the viral life cycle, results indicate that B1 could interfere with virus exit.

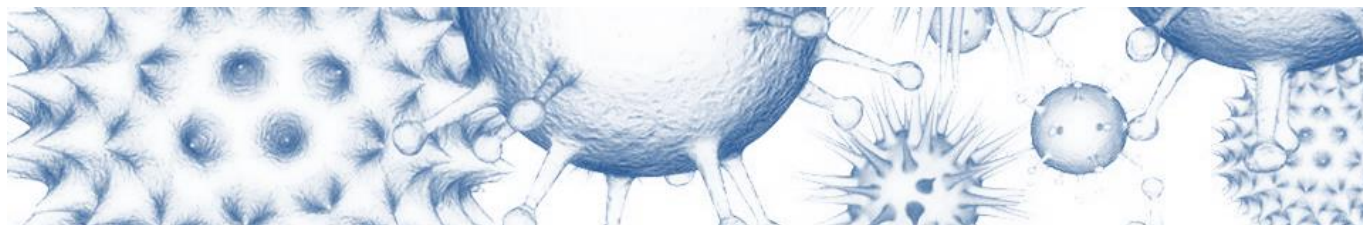
Identification and characterization of HERV-K (HML-10) in GRCh37/hg19 assembly and their possible association with the human diseases

^aMarta Cadeddu, ^aNicole Grandi, ^aLaura Vargiu[†], ^bPatricia Rodriguez-Tomé, ^cJonas Blomberg and ^aEnzo Tramontano

^aUniversity of Cagliari, Italy; ^bCRS4, Pula (Cagliari), Italy; ^cUppsala University, Sweden.

Human genome contains around 3300 Endogenous Retroviruses including the HML1-10 clade. Currently, the sole identified HML-10 sequence, HERV-K(C4) inserted within the human complement C4 gene in an antisense orientation, has been hypothesized to be involved in modulation of C4 expression, defense against retroviral infections and protection against autoimmune diseases.

Using the RetroTector software on the human genome GRCh37/hg19 assembly we identified classified and characterized 10 HML-10 sequences in chromosomes 1, 6, 16, 19 and Y and showed they have the Rec sequence. Five of them have both LTRs, 4 have only 3'-LTR and 1 is without LTRs. Age estimation analysis indicated that they inserted into the genome >25 Mya ago. Five HML-10s conserved the primer binding site region recognizing the Lys tRNA. Translational analysis showed that all proviruses have multiple stop codons that preclude their production of functional proteins. Phylogenetic analyses were performed with complete DNA sequences as well as with the Pol amino acid sequences and will be presented. Precise knowledge of all HML-10 sequences will allow further investigation of their physiological and pathological roles



Mechanism study of baicalein activity against dengue virus replication

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Dengue is among the most widespread mosquito-borne infectious diseases. It is endemic in many tropical and sub-tropical parts of the world and is rapidly spreading to other countries where the vectors are found. Dengue is caused by dengue virus (DENV), a flavivirus belonging to the Flaviviridae family. There are four distinct DENV genotypes, DENV-1, DENV-2, DENV-3 and DENV-4. All four genotypes caused a wide range of illnesses ranging from a mild febrile infection, self-limited dengue fever (DF) to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).

There are a number of plant-derived compounds with potential antiviral activity. In our previous studies we have demonstrated that some flavonoids such as fisetin, quercetin and baicalein exerted *in vitro* anti-DENV activities previously. Among the different tested flavonoids we have found that baicalein (C₁₅H₁₀O₅) exhibited the most potent anti-dengue activity but with unknown mechanism of action. Therefore this study is concentrating in the mechanisms of action of baicalein to find out the pathway of this compound against dengue virus.

DENV-2 RNA copy number was measured using the quantitative RT-PCR method after treatment with Baicalien different concentration. In this study we used TEM microscope to find out the autophagy and virus entry to the Huh7.5 cell line. The BHK dengue replicon cell line used to see if baicalein has any effect through replication cycle.

In post adsorption assay, baicalein exhibited potent antiviral activity against DENV-2 with IC₅₀ = 6.46 µg/mL. The TEM electron microscopy of baicalein has showed that baicalein can inhibit the autophagy and virus entry compare to +ev control. The BHK dengue replicon treated with baicalein showed that this compound can inhibit RNA replication of dengue virus with IC₅₀ = 11.69 µg/ml.

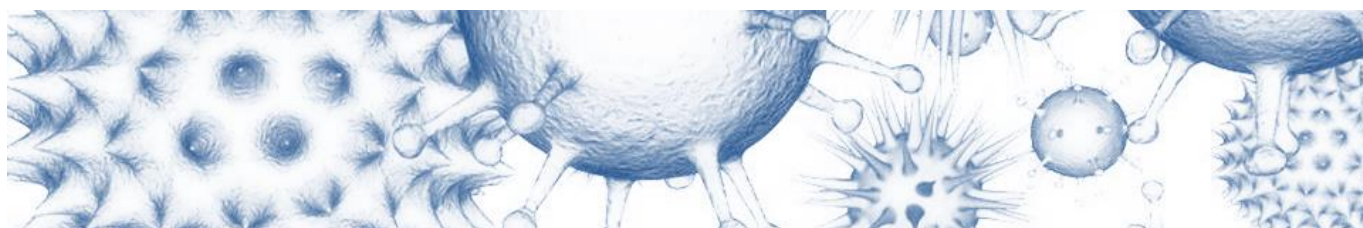
Our results showed that baicalein inhibits the dengue virus genome replication and maybe through more pathways that should be further investigated using methods such as western blotting and cytokine array.

The lipid kinase sphingosine kinase 2 is an essential host factor recruited by Chikungunya virus during infection

St. Patrick Reid, Sarah R. Tritsch, Krishna Kota, Chih-Yuan Chiang, Michael D. Ward, Sina Bavari

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The sphingosine kinases (SKs), SK1 and SK2 are the key enzymes involved in sphingosine-1 phosphate (S1-P) production and have important roles in a wide array of biological processes. Recently, SK1 has been implicated to play a role during viral infections, indicating that these kinases are likely essential host factors for viruses. By and large our understanding of SK function stems from our knowledge of SK1, in contrast, relatively little is known about SK2. In the current study, we identify SK2 as an essential host factor for Chikungunya virus (CHIKV) infection. Targeting of SK2 through the use of siRNAs or small molecule inhibitors significantly inhibited viral infection. We also observe that upon infection SK2 is dynamically relocalized to discrete puncta in infected cells. Further analysis revealed that SK2 was relocalized to the viral replication complex, and the viral protein NSP3 was required for relocalization, indicating that SK2 plays a role in viral replication. These data demonstrate that SK2 is a novel CHIKV host factor required for viral replication.



From natural products to HIV-1 IN/LEDGF interaction inhibitors: computational and synthetic approaches.

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Acquired immunodeficiency syndrome (AIDS), caused by HIV-1, is an immunosuppressive disease resulting in life-threatening opportunistic infections and malignancies. Continuous advances made in antiretroviral therapy have transformed AIDS from a rapid and lethal infection into a chronic condition, that can be controlled for many years through combination therapies with different classes of antiviral drugs. However, lifelong multidrug regimes, toxicity and emerging drug resistance making it apparent that discovery of new medicinal agents capable of specifically inhibiting HIV is urgently needed. In 2003, lens epithelium-derived growth factor p75 (LEDGF/ p75) was identified as an essential cofactor for the integration in HIV-1 replication cycle, thus emerging as an interesting approach for the development of new antiretroviral agents. At the same time, biodiversity of the plant kingdom has always provided a source of new drug candidates for almost all disease areas. Particularly, over the past decade the number of compounds exhibiting anti-HIV activity isolated from natural sources is increasing steadily. So, we have reported the application of a structure-based virtual screening workflow for the discovery of natural lead structures able to inhibit the protein-protein interaction (PPI) between HIV-1 IN and LEDGF/p75.1 Starting from these encouraging results, new series of PPI derivatives were designed and novel synthetic approaches have been planned and performed. The anti-HIV activity and the IN-LEDGF/p75 interaction inhibitory effects of the final compounds have been evaluated.

Literature:

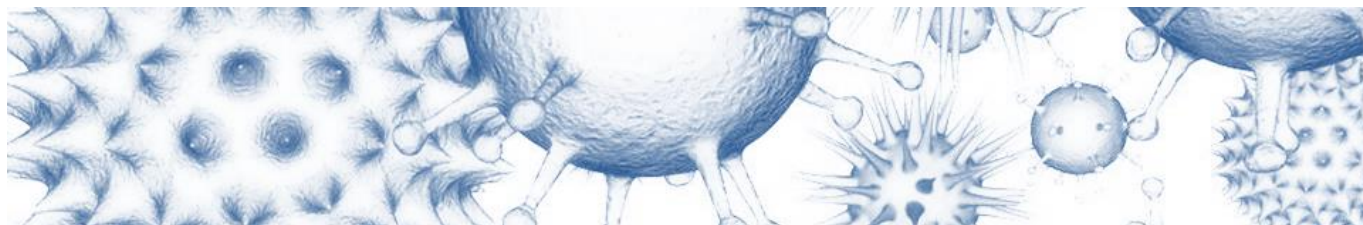
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Unrevealing the molecular mechanisms accounting for the FIV envelope glycoprotein ability to overcome Feline Tetherin restriction

Michele Celestino, Arianna Calistri, Giorgio Palù and Cristina Parolin

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Humans and other mammals have evolved different antiviral factors to defend themselves from retroviral infection. Among these, the so-called “host restriction factors” are host cellular proteins constitutively expressed or induced by interferon in response to viral infection. The species-specific expression and activity of these cellular factors limit viral host tropism and constitute a barrier to cross-species transmission events. In order to efficiently replicate, retroviruses need to overcome restriction factors and have thus evolved countermeasures or strategies to antagonize them. Recently it has been reported that Tetherin (BST2) is the cellular protein that blocks the particle release of some enveloped viruses. In this context, we focused on the study of the Feline Immunodeficiency Virus (FIV), a non primate lentivirus which causes an immunodeficiency syndrome in domestic cats that is reminiscent to AIDS in humans. We identified the feline orthologue of the human protein BST2/tetherin, renamed cBST2 or feline tetherin, and showed that it impairs HIV-1 and other lentiviruses particle release. Moreover, we identified in the FIV Envelope glycoprotein the predominant viral gene product by which FIV counteracts tetherin restriction. Starting from these findings, we start to dissect the molecular mechanisms underlying FIV ability to overcome cBST2. Our results might contribute to the elucidation of cellular mechanisms relevant for tetherin antagonism in the viral pathogenesis.



Identification of VP35 dsRNA binding pocket useful residues for development of small-molecules inhibitors against Ebola virus

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RNA viruses present dsRNA as essential intermediate in their replication cycle, which is promptly recognized as a hallmark of infection by cytosolic RIG-I-like receptors (RLRs) to induce the alpha-beta interferon (IFN- α/β) innate immune antiviral response. To escape such host defences, highly lethal Ebola virus (EBOV) prevents its detection by RLRs through the dsRNA binding properties of the VP35 protein, a viral polymerase co-factor and a determinant of EBOV virulence and pathogenesis. Hiding RLRs recognition sites on dsRNA by binding to phosphate backbone and capping dsRNA ends, EBOV VP35 suppresses the host IFN- α/β production, whereas failure in this function are associated to loss of EBOV virulence and IFN-inhibiting capabilities. By alanine scanning site-directed mutagenesis on a full length rVP35, we have characterized the importance of residues within the VP35 highly-conserved dsRNA end-capping subdomain, such as F239, Q274, I278, Q279, K319, R322 and K339. As shown by protein thermal shift and magnetic pull down assay, alanine substitution of selected residues dramatically decreases dsRNA binding. To further evaluate the impact of alanine substitution on dsRNA binding, we solved the crystallographic structure of the C-terminal dsRNA binding domain of VP35 I278A mutant. In addition, we have tested the ability of mutants residues to inhibit the IFN response compared to the VP35 wild-type, through a luciferase reporter assay system that evaluates the IFN- β production into the human lung A549 epithelial cell line. We have therefore defined the VP35 dsRNA binding pocket residues that may serve as target for the development of small-molecule inhibitors against EBOV, whose deadly haemorrhagic disease has yet to be countered by effective antiviral therapeutics.

Antiviral activity of leaves and cones of *Alnus sieboldiana* Matsum against HHV-1

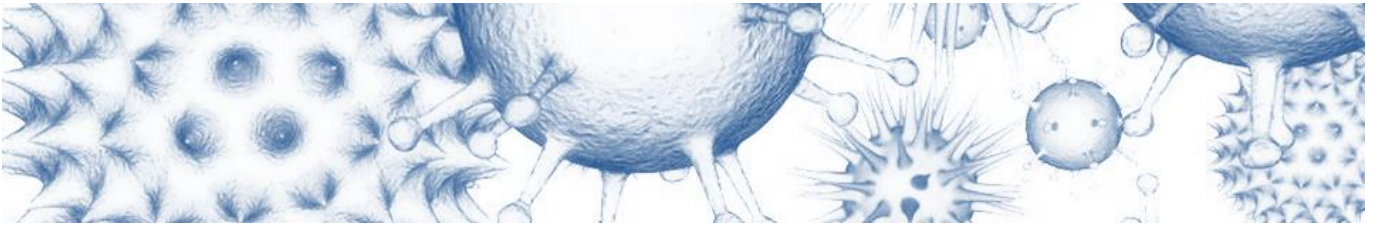
^aŁukasz Świątek, ^aBarbara Rajtar, ^bAgnieszka Ludwiczuk, ^aMałgorzata Polz-Dacewicz

^aDepartment of Virology, Medical University of Lublin, Poland

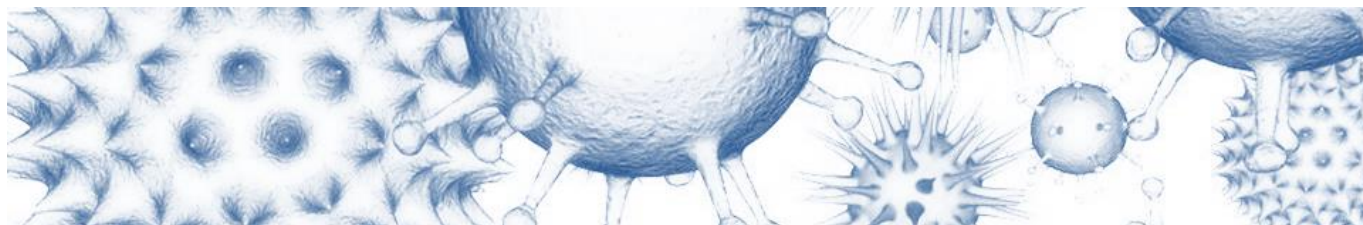
^bDepartment of Pharmacognosy, Medical University of Lublin, Poland

Activity of extracts and isolated fractions obtained from the leaves and cones of *Alnus sieboldiana* collected in Osakatoge (Shikoku Island, Japan) against HHV-1 was evaluated. After establishing of non-toxic concentrations of tested samples their influence on viral replication cycle was tested. Firstly, the VERO cells seeded in 48-well plates were pre-incubated with HHV-1 for the period of one hour (except for those left as a negative control) and then tested samples were added. The plates were incubated until the CPE was observed in the positive control (cells infected with HHV-1 and supplemented only with culture media). Subsequently, the virus titre was measured in 96-well plates with the use of end-point dilution assay. The EC₅₀ values were established with the aid of MTT tetrazolium assay and calculated with Gen5 software. Furthermore, the Real-Time PCR was used to determine the amount of viral DNA in samples.

Methanol extract from leaves (MEL), in the concentration of 150 and 190 $\mu\text{g/ml}$ and buthanol fraction from the cons extract in the concentration of 100 $\mu\text{g/ml}$ completely blocked the replication of HHV-1. In case of 100 $\mu\text{g/ml}$ MEL virus titre was reduced by 3.19 log suggesting dose-response relationship between concentration of MEL and virus inhibition. Potent antiviral activity was also noted for methanol extract from cones (120 $\mu\text{g/ml}$) and buthanol fraction from leaves extract (150 $\mu\text{g/ml}$) which reduced virus titre by 2.56 and 3.54 log, respectively. The lowest antiviral activity was observed in case of ethyl acetate fractions from both cones and leaves methanol extracts. The results of Real-Time PCR confirmed antiviral activity of selected samples.



Poster abstracts



N°1

New rationally designed dual inhibitors of both HIV-1 RT associated functions

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The HIV-1 Reverse Transcriptase (RT) is a validated and deeply explored biological target for the treatment of AIDS. However, only drugs targeting the RT-associated DNA polymerase (DP) function have been approved for clinic use. RNase H function is essential for the reverse transcription but, until now no inhibitors, specific for this enzymatic activity have been introduced in therapy. Nevertheless, quite recently, some RNase H inhibitors (RNase HI) have been designed and studied. Generally they act by chelating the Mg²⁺ ions within the active site, however some RNase H inhibitors bind the RT in an allosteric site located between polymerase catalytic region and NNIBP 50 Å from the RNase H catalytic site. Starting from an allosteric RNase H inhibitor, by virtual screening methods, we have discovered a series of molecules which exhibit dual inhibitory activity capable of inhibiting both RT associated functions. Therefore, we focused on the design of new dual inhibitors exploring several optimization paths towards more potent compounds. Here we report on some new, rationally designed, compounds that exhibit dual inhibitory properties towards both HIV-1 RT associated functions.

N°2

High-throughput screening system(s) for the identification of antivirals against African Swine Fever Virus, Influenza Virus, and Pseudorabies Virus

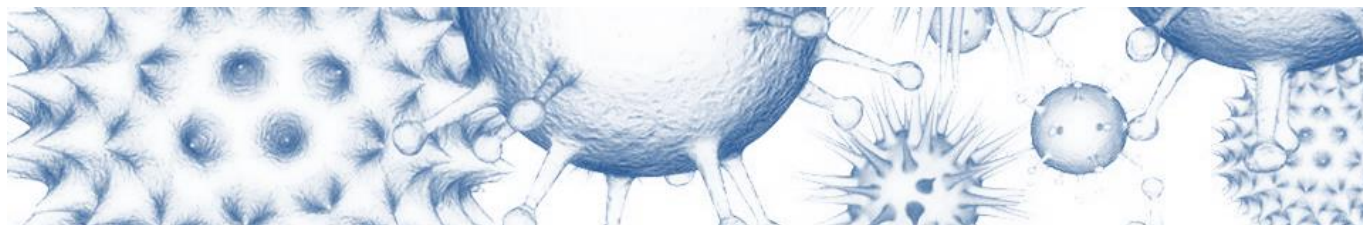
Jessica Bogs, Günther Keil, Donata Hoffmann, Walter Fuchs, Thomas C. Mettenleiter

Friedrich-Loeffler-Institut, Greifswald – Insel Riems

Background: The lack of new, innovative antivirals is one of the major challenges of infection control in the future. This is especially true with regard to infectious diseases against which no vaccines are available to prevent outbreaks (current example in veterinary medicine: infections with African Swine Fever Virus in the EU) and to infectious diseases with a constantly increasing number of resistant strains (current example in human medicine: infections with Influenza A Viruses with resistance to NA inhibitors or M2 blockers).

Methods: Lead substances (and derivatives) are provided by a partner from the field of natural product research. Some of these compounds were proven to have antimicrobial and/or antifungal activities *in vitro*, but have never been tested for any antiviral properties. Therefore, recombinant viruses of different virus families (African Swine Fever Virus: *Asfarviridae* (dsDNA), cytoplasmic replication; Influenza A Virus: *Orthomyxoviridae* ((-)ssRNA), nuclear replication; Pseudorabies Virus: *Herpesviridae* (dsDNA), nuclear replication) expressing the green fluorescent protein (GFP) will be utilized for the assays, enabling a fluorescence-based evaluation of antiviral activities of the compounds. Different cell lines have to be analysed with regard to their suitability for the screening. Lastly, an automated high throughput system for a rapid and reliable screening of a wide range of natural products will be established.

Importance: The identification of substances with proven efficacy in *in vitro* tests leads to a prioritized *in vivo* testing and, therefore, paves the way for the introduction of new compounds into pharmaceutical pipelines.



N°3

Virtual screening and in-vitro validation of novel inhibitors of influenza virus PA endonuclease

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Vaccination and antiviral agents are the primary measures against human influenza viruses. However, both interventions have shortcomings, and hence, there is an urgent need to develop new and more efficacious anti-influenza virus drugs. The influenza virus RNA-dependent RNA polymerase complex (RdRp), a heterotrimeric protein complex that is responsible for viral RNA transcription, represents an unexplored target for antiviral drug development. One particularly attractive approach is interference with the endonucleolytic “cap-snatching” reaction by the PA subunit of the RdRp, more precisely by inhibiting the metal-dependent catalytic activity which resides in the N-terminal part of PA (PA-Nter).

In the last two decades, several small molecule PA inhibitors (PAIs) have been discovered. Among them, compounds belonging to the class of substituted 2,4-dioxobutanoic acids were identified as particularly potent and selective PAIs in both enzyme and cell-based assays. A few other classes of potential PAIs have been identified. All these diverse compounds bear distinct pharmacophoric fragments with chelating motifs able to bind the bivalent metal ions in the catalytic core of PA-Nter. More recently, the availability of crystallographic structures of PA-Nter has enabled rational design of PAIs with improved binding properties.

Here, we present a new coupled pharmacophore/docking virtual screening approach that allowed us to identify novel PAIs having interesting inhibitory activity in a PA-Nter enzymatic assay, as well as antiviral activity in a cell-based influenza virus yield assay.

N°4

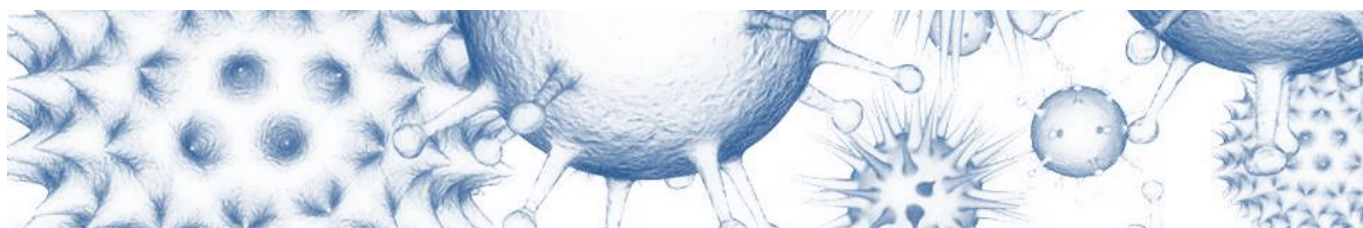
Genotypic and phenotypic analysis of Gag and Pol polyproteins mutations selected by antiretroviral therapy

Ilaria Carli, Claudia Del Vecchio, Saverio G. Parisi, F. Dal Bello, Arianna Calistri, Giorgio Palù, Cristina Parolin

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The Human Immunodeficiency virus type 1 (HIV-1) protease (PR) and reverse transcriptase (RT) are key enzymes in viral replication and major target of antiretroviral therapy. The mechanisms of resistance mainly involve mutations altering the interaction of viral enzymes and inhibitors. Recent studies reveal that, besides the enzymes-encoding ones, other regions might contribute to development of resistance. In particular, some specific cleavage and non-cleavage site mutations in Gag increase the replication ability of mutant viruses. The role of amino acid substitutions in different Gag domains selected in vivo, remains to be elucidated. To clarify this aspect, we analyzed clinical samples of HIV-1 infected patients failing PR Inhibitors (PIs) and RT Inhibitors (RTIs) treatment among a cohort of five infectious diseases units located in Veneto in northeastern Italy.

Plasma and PBMCs samples were used for gag and pol genes PCR amplification and sequencing. Besides polymorphisms and multiple amino acid substitutions associated with inhibitors resistance, genotypic analyses have identified insertions within the Gag at the level of the matrix domain and the p6 domains. The Gag-encoding region derived from selected patients was adopted to reconstitute recombinant HIV-1 viruses in an otherwise wildtype background (HIV-1 LAI). The effect of these mutations have been examined in terms of Gag and Gag-Pol processing, particle release and viral replication. Our results would contribute to better characterize the role of Gag and the relations with PR and RT in resistance development, their relevance in viral replication and evolution in the presence or in the absence of drugs.



N°5

2-methoxynaphthalen thiazole derivate: a new HIV-1 RT Ribonuclease H/DNA polymerase dual inhibitor active on viral replication

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Since the discovery of 3'-azidothymidine, the HIV-1 coded reverse transcriptase (RT) has been the main target for drug treatments that successfully turned the lethal progression of the acquired immunodeficiency syndrome into a chronic disease. However, emergence of long term therapy side effects and selection of drug resistant viral strains still demand novel anti-HIV agents. HIV-1 RT performs viral genome replication combining two functions, both essential for viral replication: DNA polymerase (DP) and ribonuclease H (RNase H). Dual allosteric inhibitors of both RNase H and DP are a fascinating possibility to overcome the selection of drug resistant viruses. Currently, none of the approved antiviral drugs acts on both activities. In a previous virtual screening a number of scaffolds for dual functions RT inhibitors were identified, among them a naphthalene derivate, that was further developed into a series of 12 compounds and studied as a promising dual inhibitor. 12 new compounds inhibited both HIV-1 RT functions in the low micromolar range. Among them derivative AR3f inhibited the RT-associated RNase H and DP activities with IC₅₀ values of 6 and 12 μ M, respectively, and it was also able to inhibit viral replication in cell-based assay (EC₅₀ 15 μ M) with no cytotoxic effects. Mode of action studies, performed by single-site mutagenesis experiments, revealed that AR3f binds to the NNRTI binding pocket, but its inhibition pattern is different from the classical non-nucleoside RT inhibitors. It retained full potency of inhibition against Y181C RT while decreased its potency of inhibition on the K103N RT. Results showed that AR3f binds also to a second pocket close to the RNase H active site. In fact substitution of two amino acid residues in the RNase H domain affected its potency of inhibition of the sole RNase H function. AR3f indeed was completely ineffective in inhibiting N474A RT RNase H, while was fully efficient against Its DP function. The new naphthalene-derivative AR3f was shown to inhibit both RNase H and DP RT-associated functions in the low micromolar range, and to inhibit viral replication in cell culture. Site-directed mutagenesis studies supported the hypothesis that AR3f could bind to two different RT sites, and act as a dual site dual inhibitor.

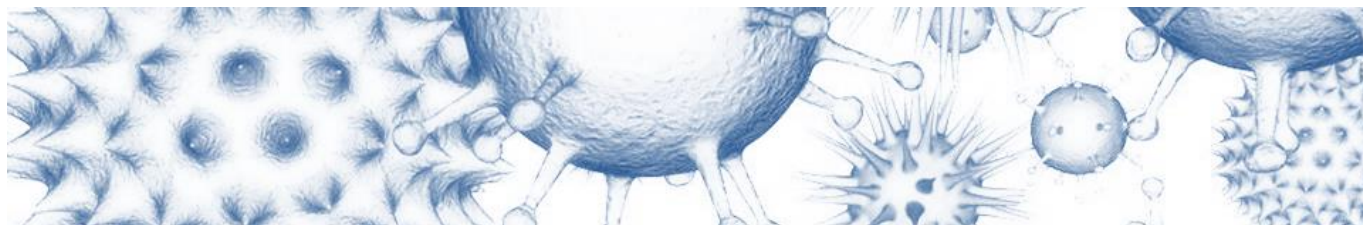
N°6

Identification of highly conserved residues involved in the inhibition of the HIV-1 integrase by diketoacid derivatives

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The catalytic site of the human immunodeficiency virus type 1 (HIV-1) integrase (IN) contains a central core composed by three highly conserved amino acid residues, forming the DDE motif, that coordinate two divalent metal ion Mg²⁺. Diketo acid (DKA) derivatives are among the first compounds reported to interact with the Mg²⁺ cofactors in the IN active site. Recently, a series of pyrrolyl DKA derivatives was identified to inhibit HIV-1 IN and a few compounds were chosen as chemical tools to investigate their interaction with the IN catalytic site. Docking studies suggested the possibility that these DKAs could interact with 143, 145, 146 and 148 residues. Hence, we performed site-directed mutagenesis on these residues and on residues involved in IN resistance to Raltegravir (Y143A and N155H). Among tested DKA, RDS1644 that showed an IN IC₅₀ value of 0.3 μ M was fully effective against both Y143A and N155H INs while its efficacy decreased by > 22- and 9-folds when tested on E138K/Q148R and P145A INs. These data provide important insights for the rational design of IN inhibitors.



N°7

Isolation and characterization of Nanobodies against Junin virus as new and improved treatment option for Argentine hemorrhagic fever

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Argentine Hemorrhagic Fever (AHF) is an endemic disease specific of the central part of Argentina with a case-fatality rate, without treatment, ranging from 15% to 30%. Although the use of a live attenuated vaccine Candid#1, has markedly reduced the incidence of AHF in the population at risk, supportive therapy remains very important in the management of patients with AHF.

In this work we explored the development of a novel and improved therapeutic intervention against AHF, based on the generation and use of single domain antibodies (also known as Nanobodies or VHH). This technology combines unparalleled antigen-targeting specificities with ease of expression, purification and re-engineering opportunities.

The generation of the nanobodies directed against JUNV was performed by the immunization of an alpaca with purified UV-inactivated Candid#1 JUNV. After immunization, a cDNA library was generated and subsequently used to isolate phages that express JUNV-binding nanobodies at their surface by biopanning. The JUNV-binding capacity of these VHHs was evaluated by ELISA from the periplasmic extracts and the neutralizing activity of these binders was then evaluated functionally by a virus neutralization assay against JUNV Candid#1 strain. Results showed that 4 of the 70 candidates were able to neutralize successfully Candid#1 JUNV strain replication at very low concentrations. These findings constitute the first demonstration that the nanobody technology could be used to avoid JUNV replication. Afterwards testing of the virus-neutralizing activity against pathogenic strains will allow demonstrating the potential use of this technology treat and potentially preventing arenavirus hemorrhagic fever in human.

N°8

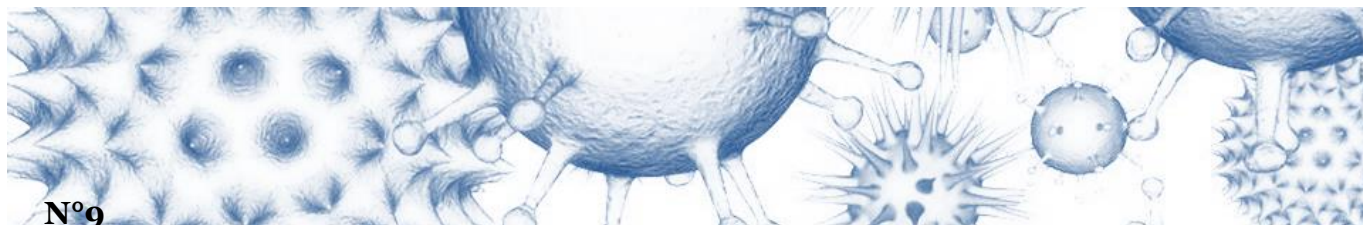
New inhibitors of ribonuclease H function of the HIV-1 reverse transcriptase enzyme

^aV. N. Madia, ^aL. Pescatori, ^aG. C. Crucitti, ^aA. Messore, ^aG. Pupo, ^bE. Tramontano, ^bA. Corona, ^bF. Esposito, ^aR. Costi and ^aR. Di Santo

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The HIV-1 RT has two associated activities: the DNA polymerase activity and the RNase H activity that selectively degrades the RNA strand of the hybrid RNA/DNA formed during the synthesis of the minus (-) strand DNA that uses (+)RNA as a template. Several studies have demonstrated that the abolition of the HIV-1 RNase H function stops the virus replication. Therefore, it is a validated and attractive target for the development of new anti-retroviral agents. Despite this, it has been little explored and it needs to be further developed through the support of new HIV/AIDS drug discovery programs, in order to identify more efficient anti-HIV drugs that could be used for therapy.¹⁻⁵

The RT inhibitors currently approved for the treatment of HIV infection inhibit the RT polymerase activity, while none of them block the RT RNase H activity. Until now, only a few compounds have been described to inhibit the HIV-1 RNase H function. Among them aryldiketo acid derivatives proven to inhibit both integrase enzyme and RNase H function of the RT. Pursuing our studies on pyrrolyl DKA derivatives as dual inhibitors of IN and RNase H we developed non DKA scaffold and found a new class of compounds that selectively inhibited the RNase H. The data coming from the biological assays will be shown and discussed.



N°9

Design of novel HIV-1 Integrase inhibitors

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HIV-1 Integrase (IN) is an essential enzyme for HIV-1 replication and catalyzes the integration of viral DNA into the host cell genome. This latter process is realized in two spatially and temporally distinct steps: 3'-processing and strand-transfer. The first step occurs in the cytoplasm, where IN binds the viral DNA and then cleaves a highly conserved dinucleotide sequence (CA) from the 3' end of each strand. Then the protein-DNA complex binds several cofactors and is transported into the nucleus. After nuclear import, two DNA-bound dimers approach each other in the presence of the cellular protein LEDGF/p75 and form a tetramer which catalyzes the strand-transfer process.

The free IN tetramer does not bind DNA directly, and tetramerization occurs only by the interaction between two DNA-bound dimers. Today three IN inhibitors are approved by the FDA: Raltegravir, Elvitegravir and Dolutegravir. These drugs exert their effect by binding to the active site. Despite their excellent safety profile and efficacy, INSTI-resistant strains have already emerged in the clinic, underscoring the importance of developing next-generation IN inhibitors.

Herein many different computational methods have been used in order to identify novel IN inhibitors active against INSTI-resistant mutants. To this aim, three different allosteric binding pockets were explored [1-3] by the use of computational tools. Furthermore, virtual screening approaches consisting of docking studies, pharmacophoric models and molecular dynamics were applied in order to screen several commercial databases.

1. Tintori C et al *Bioorg. Med. Chem. Lett.* 2012, 22, 3109
2. Wielens J et al *FEBS Lett.* 2010, 584, 1455
3. Christ F et al *Virology* 2013, 435, 102

N°10

Optimization of 2-phenyquinazolinone CDK9 inhibitors as anti-HIV-1 agents

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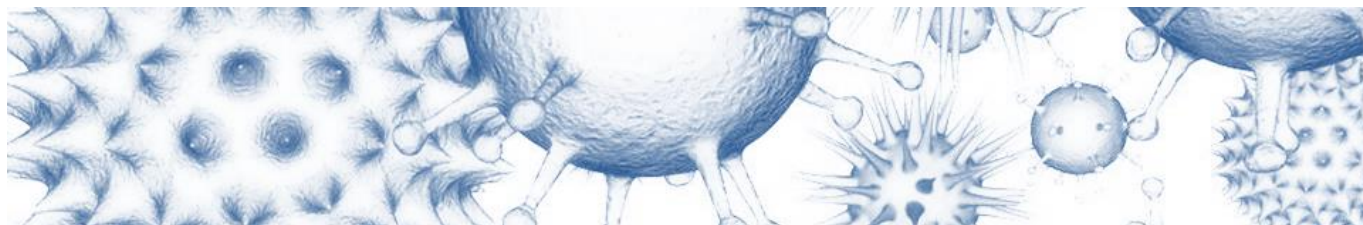
Within HIV-1 replicative cycle, the Tat-mediated transcription is a crucial step, required to re-initiate viral replication from post-integration latency and to keep the virus in circulation. It involves an intricate interplay of both viral and host cellular factors. Among the latter, the cyclin dependent kinase 9 (CDK9), which exerts its action in complex with the Cyclin T1 to give P-TEFb, is an attractive target because of its pivotal role in sustaining high levels of HIV transcription.

Aided by *in-silico* techniques, we have recently developed a new class of non-toxic anti-CDK9 inhibitors based on a 2-phenylquinazolinone scaffold.¹ CDK9 Inhibition translated into a selective interference with the Tat-mediated transcription and into the inhibition of HIV-1 reactivation from latently infected cells. Considering that the 2-phenylquinazolinones are merely fragments, they are largely optimizable into improved derivatives. Thus, computational studies were carried onto "grow" the fragments, leading to the design and synthesis of an enlarged series of analogues.

The *in-silico* and synthetic efforts along with the anti-kinase and antiviral profile of the compounds will be presented.

References

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N°11

Novel carbocyclic nucleoside derivatives as potential antiviral agents

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Modified nucleosides have provided a wealth of drug candidates and clinically approved therapeutics. The replacement of the furanose oxygen in the sugar moiety with a methylene group, yielding a class of nucleosides, known as carbocyclic nucleosides. This modification increases stability and lipophilicity to the analogues, both highly desirable traits for drugs. Potent antivirals such as Lobucavir, Abacavir and Entecavir are important members of this class of compound in the field of medicinal chemistry.

The 5'-norcarbocycles in general, possess interesting characteristics due to their increased stability and lack of 5'-phosphorylation. This latter feature is responsible for a decrease in cytotoxicity of 5'-norcarbocycles.

In an attempt to further explore therapeutic potential of 5'-norcarbocycles, a new type of carbocyclic 5'-noruridine analogues was designed based on the observation that a series of N1-substituted uracil diaryl derivatives proved to be potent non-nucleoside inhibitors of RT-HIV.

Analogue of 2',3'-dideoxy-2',3'-didehydro-5'-nor-uridine was selected for investigation. Two different aspects of the scaffold were investigated: the effects of adding a linker between the carbocyclic and phenyl fragments and introduction of different substituents on the 4'-benzoyl residue.

Analogues of 2',3'-dideoxy-2',3'-didehydro-5'-nor-uridine, bearing benzyl in N3-position and benzoyl-, 3,5-dichloro- or 3,5-dimethylbenzoyl groups in 4'-position, showed inhibitory activity against HIV-RT wild-type (Ki 5-10 µM) and mutants L100I (Ki 1.2-2.1 µM) and K103N (Ki 8-17 µM).

N°12

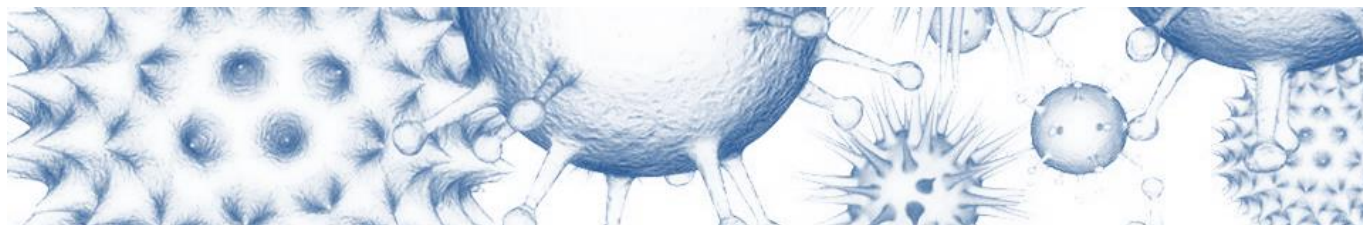
Oseltamivir and Zanamivir susceptibility profile of human influenza viruses circulating in Italy from 2012/13 to the 2013/14 seasons

Simona Puzelli, Marzia Facchini, Monica Meola, Angela Di Martino, Concetta Fabiani, Palmieri Annapina, Laura Calzoletti, and Isabella Donatelli

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Antiviral drugs represent one of the most important tools for the control of influenza epidemics and pandemics. Currently, the neuraminidase inhibitors (NAIs) are the only antiviral agents effective against influenza infections, due to the globally rapid spread of Adamantanes-resistant virus variants. It is therefore important to monitor the susceptibility of the clinical isolates to NA inhibitors and to detect the emergence of resistant strains. Here, we report the results of a surveillance study on the sensitivity of human influenza A and B viruses isolated in Italy during the last two seasons to the most common NAIs, Oseltamivir and Zanamivir.

A total of 227 isolates belonging to different types and subtypes (95 A/H3N2, 81 A/H1N1pdm09 and 51 B) were tested for their susceptibility to both oseltamivir and zanamivir, by using a NA activity inhibition assay (MUNANA) in combination with NA sequence analyses. All A and B viruses tested by MUNANA assay were sensitive to both NAIs. Differences in the mean IC₅₀ values for oseltamivir and zanamivir were noted according to subtype and NAI. Particularly, the A/H1N1pdm09 viruses demonstrated a higher level of sensitivity to zanamivir than to oseltamivir (mean IC₅₀: 0.48 nM and 1.4 nM, respectively); B viruses were even more sensitive to zanamivir (mean IC₅₀: 3.7 nM) than to oseltamivir (mean IC₅₀: 40 nM), whereas similar IC₅₀ values for both drugs were found for the H3N2 isolates (mean IC₅₀: 0.46 nM for oseltamivir versus 0.35 nM for zanamivir). None of the NA sequences analyzed presented mutations typically associated with oseltamivir and zanamivir resistance.



N°13

Discovery of 4(1*H*)-Quinolinone Derivatives as Anti-Chikungunya Virus Agents.

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Chikungunya is one of the most important re-emerging human arboviral infections of global significance. Chikungunya virus (CHIKV) is an arthropod-borne virus (arbovirus) and is transmitted to human primarily by *Aedes aegypti* mosquitoes, the infamous yellow fever propagator.^{1,2} The symptoms generally start 4–7 days after the bite. Acute infection lasts for 1–10 days and is characterized by abrupt onset of fever, headache, fatigue, nausea, vomiting, rash, myalgia, and severe arthralgia. Despite the gravity of its infectious potency, there is currently no antiviral treatment or vaccine against CHIKV infection. Previous studies have reported anti-CHIKV activities in vitro for some compounds.^{3–5} Among these, only chloroquine has been tested in vivo but with poor results. For these reasons, there is an urgent need for the discovery of antivirals active against CHIKV infection. For many years we focused our research in the field of anti-infective agents, in particular to find compounds active against HIV,⁶ HCV,⁷ and influenza A.⁸ Recently, we undertook a screening for the identification of novel anti-HCV agents and widened the test with the aim to discover compounds able to block the CHIKV replication. The preliminary data will be shown and discussed.

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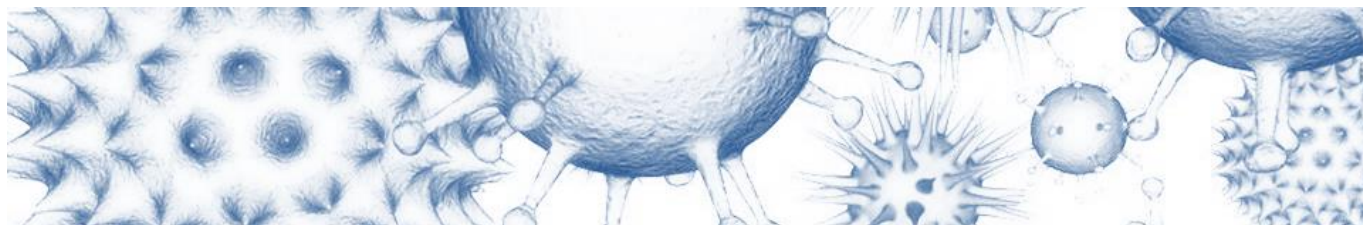
N°14

Binding Free Energy Based Structural Dynamics Analysis of HIV-1 RT RNase H-Inhibitor Complexes

Vasanthanathan Poongavanam

Department of Physics, Chemistry and Pharmacy University of Southern Denmark

Accurate prediction of binding free energies associated with small molecules binding to a receptor is a major challenge in drug design processes. To achieve this goal many computational methods have been developed ranging from highly efficient empirical based docking schemes to high accuracy methods based on e.g. free energy calculations. In this study, binding affinity predictions for a set of HIV-1 RNase H inhibitors have been performed using MM-PB(GB)/SA methods. The current study describes in detail how the choice of initial ligand structures, e.g. protonation states, impacts the predicted ranking of the compounds. In addition we study the structural dynamics of the RNase H complexes using molecular dynamics. The role of each residue contribution to the overall binding free energy is also explored and used to explain the variations in the inhibition potency. The results reported here can be useful for design of small molecules against RNase H activity in the development of effective drugs for HIV treatment.



N°15

Design and discovery of substituted pyrazole and pyrimidine scaffolds as novel class of NNRTIs

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Drug Design & Discovery Laboratory, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad, India

A novel series of pyrazole and pyrimidine hybrid compounds has been designed using the molinspiration toolkit based on Lipinski's rule of five and developed via sequential reactions involving diazotization, active methylation and cyclisation. These molecules have been subsequently tested for anti-HIV activity using TZM-bl cell lines along with Luciferase expression profile of the TZM-bl cells after infecting with NL4.3 virus along with MTT assay for the cytotoxicity determination.

In Anti-HIV assay, molecules having pyrazole amine with distant position of electron withdrawing group showed 91-98 % inhibition. Further these compounds in Luciferase assay showed considerable inhibition of infection. Cytotoxicity assay, revealed that an increase in the concentration of most active compounds from 25 mg/mL to 125 mg/mL did not appreciably lower the percentage of cell viability. On close inspection of the best docked pose of most active compound clearly establish that it attained a "horseshoe-like" conformation by interacting with Tyr181 and Tyr188 of the p66 subunit in the NNIBP. While the non-active molecule showed rigid conformation with no favourable interaction. Hence, it was inferred that target molecules showed potent anti-HIV activity, while presenting no significant toxicity at the test dosages. The antibacterial activity of the target compounds was also determined against a panel of Gram-positive and Gram-negative human pathogens where they showed prominent inhibition of bacterial organisms.

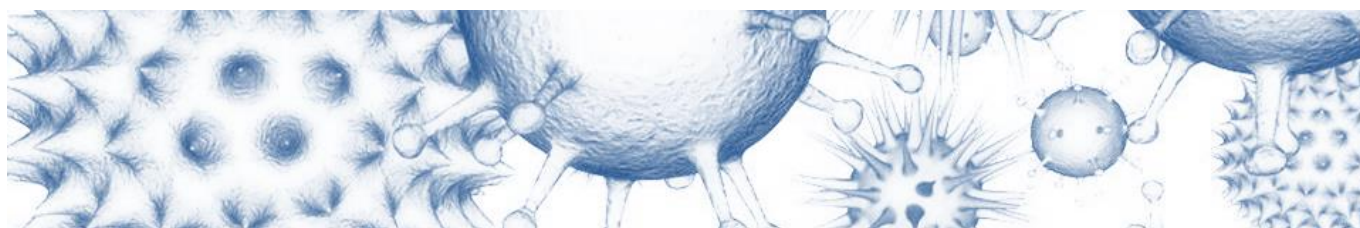
N°16

Identification of nucleotide Mutations in the thymidine kinase genes in clinical herpes simplex virus type 1 isolates.

Axel Schubert and Thomas Mertens

Institute of Virology, German Reference Laboratory for CMV, University Hospital Ulm, Ulm, Germany

The role of specific mutations in the thymidine kinase gene (TK) of herpes simplex virus (HSV) for the development of different resistance phenotypes can be examined reliably by the alignment of resistance phenotype and genotype. In this study, clinical HSV-1 isolates obtained between 2004 and 2011 from 26 different patients with peripheral blood stem cell transplantation were examined in parallel by both the phenotypic and genotypic resistance testing. Thirteen isolates resistant to acyclovir (ACV) had consistently resistant-related frame shift or non-synonymous mutations in the TK gene, one out of these (insertion of C at the nucleotide positions 1061-1065) was novel. Six strains, further characterized in the institute of virology and antiviral therapy at Jena University Clinic (Jena, Germany German Reference Laboratory for HSV and VZV) were phenotypically resistant to ACV, PCV, foscarnet (FOS) and, except one, sensitive to brivudin, had uniformly resistant-related substitutions in the DNA pol. Finally, 4 isolates, resistant to ACV, PCV and 2 out of these resistant to brivudin, had known but also unclear substitutions in the TK and DNA pol. In conclusion, clinical ACV-resistant HSV-1 isolates, containing resistant-associated mutations in the TK gene, can be ever regarded as cross-resistant to other nucleoside analogues such as brivudin. In contrast, clinical ACV-resistant HSV-1 strains, whose resistance is caused by mutations in the DNA pol gene, are likely cross-resistant to FOS but sensitive to brivudin. This should be considered for alternative antiviral treatment of HSV-1 infections in case of ACV resistance.



N°17

Enhanced Protection Of Lower Dosage of Oseltamivir Combined with Some Biological Response Modifiers Against Influenza Virus H3N2 Infection *in Vivo*

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Influenza is often associated with serious complications and huge medical and economical losses to the mankind. Targeting different aspects of the pathogenesis could be a reasonable approach to combat the severity of the disease. We investigated combined activity of oseltamivir – specific anti-influenza inhibitor with proved efficacy and some promising biological response modifiers as stimulants enhancing the host responses against the toxic effects of viral replication in the organism. Mice were inoculated i.n. with influenza A/Panama/2007/99 (H3N2) virus 4h after first oseltamivir dose and the course lasted for five days. The antioxidant ellagic acid (EA) was applied orally once daily for five days starting 2 hours prior to the challenge. The immune modulator isoprinosine (ISO) was administered for 10 days in two intakes the daily dosage beginning from the day of infection. Polyphenol complex (PC) from *Geranium sanguineum* was given once 3 hours pre-infection i.n. Mortality rates, protection index (PI), mean survival time (MST) and body weight changes were determined through 14 days p.i. Beneficial effects were observed of double combinations of oseltamivir of 1.25, 2.5 and 5 mg/kg with PC (2.5 and 5 mg/kg), EA (500 mg/kg) and ISO (500 mg/kg) by reduction of mortality rates and PI up to 90% for selected combinations. MST was prolonged up to 13.7 days and body weight loss was reduced. Data suggest that oseltamivir at daily doses lower than optimal effective one *in vivo* when administered in combination with antioxidants and immune modulators demonstrated enhanced protective effect against infection with influenza virus A (H3N2) in mice.

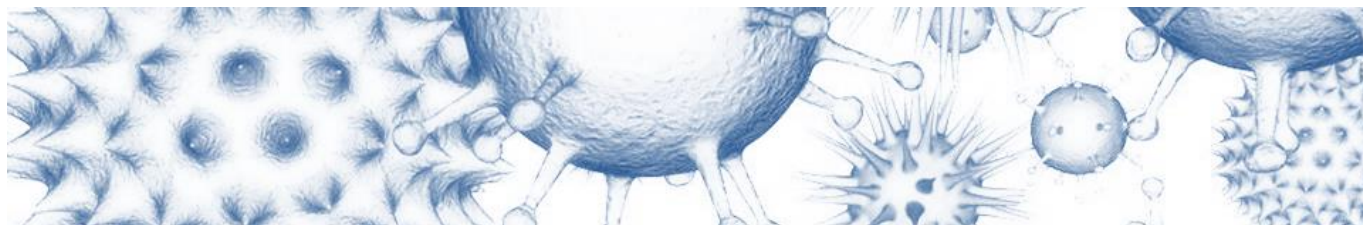
N°18

The 6-Aminoquinolone WC5 Inhibits Different Functions of the Immediate-Early 2 (IE2) Protein of Human Cytomegalovirus that are Essential for Viral Replication

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^aDept of Molecular Medicine, University of Padua, Padua; ^bDept of Life Sciences and Systems Biology, University of Turin, Turin; ^cDept of Chemistry and Technology of Drugs, University of Perugia, Perugia.

Human cytomegalovirus (HCMV) IE2 protein is a multifunctional factor essential for viral replication. IE2 modulates both viral and host gene expression, dysregulates cell cycle progression, induces pro-inflammatory effects, and antagonizes cellular antiviral responses. Based on these facts, IE2 has been proposed as an important target for the development of innovative antiviral approaches. We previously identified the 6-aminoquinolone WC5 as a potent inhibitor of HCMV replication and here we report the deeper dissection of WC5 mechanism of action against viral IE2 protein. By GST-pulldown assays, mutagenesis, cell-based assays, and electrophoretic mobility shift assays, we demonstrated that WC5 specifically blocks the interactions of IE2 with IE2-responsive sequences within at least two different viral promoters. WC5 did affect neither IE2 dimerization, nor its interaction with TBP, nor the expression of a set of cellular genes that are stimulated by IE2. In contrast, WC5 interfered with the IE2-dependent negative regulation of the Major Immediate-Early promoter activity by preventing IE2 binding to the crs element. Moreover, WC5 reduced the IE2-dependent transactivation of a series of indicator constructs driven by different portions of the Early UL54 gene promoter and also inhibited the transactivation of the murine CMV early E1 promoter by the IE3 protein, the HCMV IE2 homolog. In conclusion, these results indicate that the overall anti-HCMV activity of WC5 depends on its ability to interfere with IE2 binding to crucial responsive promoters. Importantly, this mechanism seems to be conserved in the murine CMV, thus paving the way to further preclinical evaluation in an animal model.



N°19

Engineering Of Influenza Virus-Like Particles By Expression Of Viral Structural Proteins

Marta Toffoletto, Michele Celestino, Cristina Parolin, Giorgio Palu' and Arianna Calistri.

Department of Molecular Medicine, University of Padua, Padua, Italy.

Virus Like Particles (VLPs) are extremely promising as vaccine candidates for many different viruses. VLPs mimic the three-dimensional conformation of native virus, but lack pathogenicity. VLPs can be generated by expressing specific viral structural proteins and could be used as “platforms” for the presentation of foreign epitopes. In influenza budding, M1 represents the major driving force, but the matrix protein M2 is also crucial. In fact, M2 can substitute for the ESCRT components, thus rendering MVB biogenesis pathway dispensable for influenza virus budding and allowing the direct interaction between the plasma membrane and M1. In order to optimize the production of influenza VLPs for the development of a universal vaccine, we evaluated the possibility to render M1 budding independent from M2. With this aim, we generated different recombinant M1 proteins capable of interacting with different ESCRT components and/or to directly contact the plasma membrane. On the other hand, we investigated the role of M2 as a substitute for ESCRT components using specific mutants of the feline immunodeficiency virus (FIV) Gag protein. Finally, in order to induce a broad immune response, we also generated different haemagglutinin (HA) mutants characterized by the absence of the globular head (Headless HA) and we analysed their ability to be incorporated into engineered VLPs. Overall our data provide important biological insights into the function/characteristics of the influenza virus M1 and M2 proteins and useful information for the development and improvement of influenza-based VLPs to be employed in vaccination strategies.

N°20

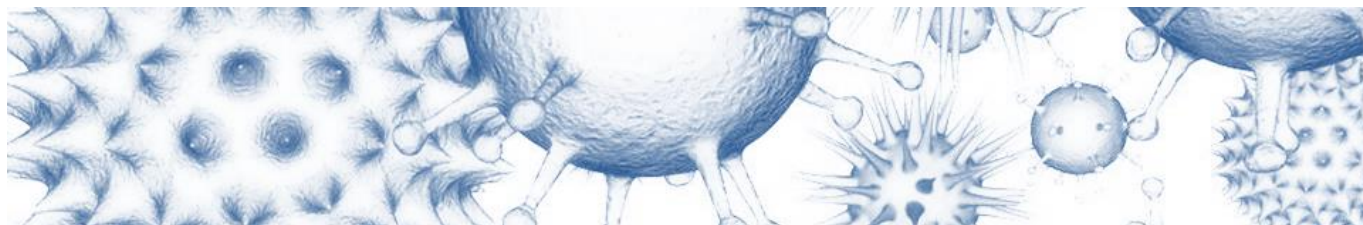
Screening of a cell inhibitor library for antiviral compounds against coronaviruses

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The emergence of MERS-CoV in 2012 has highlighted the need for developing antivirals against coronaviruses. Here we describe a study aiming at recognizing novel compounds with potential broad spectrum antiviral efficacy against coronaviruses. We have at our disposal a library of over 2000 inhibitors directed against different host cell functions, which we will screen for compounds that reduce replication of the low pathogenic hCoV-229E coronavirus. A simple commercial test for measuring cell death will be utilized in the initial screen and positive hits will be further analysed for efficacy against highly pathogenic MERS-CoV and SARS-CoV in BSL-3 facilities. Similar approaches previously with influenza A and measles viruses have proved successful, so we expect to identify at least a couple of potential antiviral compounds. Besides identifying antiviral compounds, our work will also give information about the host cell mechanisms being utilized by coronaviruses.



N°21

Metal-chelating 2-Hydroxyphenyl Amide Pharmacophore for Inhibition of Influenza Virus Endonuclease

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The influenza virus PA endonuclease is an attractive target for development of novel anti-influenza virus therapeutics. Reported PA inhibitors are assumed to chelate the divalent metal ion(s) in the enzyme's catalytic site, which is located in the N-terminal part of PA (PA-Nter). In this work, a series of 2-hydroxybenzamide-based compounds have been synthesized and biologically evaluated in order to identify the essential pharmacophoric motif, which could be involved in functional sequestration of one or both divalent metal ions (probably Mg^{2+}) in the catalytic site of PA. By using HL_1 , H_2L_2 , and HL_3 as model ligands with Mg^{2+} ions, we isolated and fully characterized a series of complexes and tested them for inhibitory activity towards PA-Nter endonuclease. H_2L_2 and the corresponding Mg^{2+} complex showed an interesting inhibition of the endonuclease activity. The crystal structures of HL_1 , H_2L_2 , and the magnesium complex $[Mg(L_3)_2(MeOH)_2] \cdot 2MeOH$ were solved by X-ray diffraction analysis. Furthermore, the speciation models for HL_1 , H_2L_2 , and HL_3 with Mg^{2+} were obtained and the formation constants of the complexes were measured. Preliminary docking calculations were conducted to investigate the potential interactions of the title compounds with essential amino acids in the PA-Nter active site. These findings supported the "two-metal" coordination of Mg^{2+} by a donor triad atoms chemotype as a powerful strategy to develop more potent PA endonuclease inhibitors.

N°22

Studies on Innate Immunity in Primary Woodchuck Hepatocytes Following Treatment with Lamivudin and Interferon

Marta G. Murreddu, Manasa Suresh, Jummanna H. Hussain, Stephan Menne

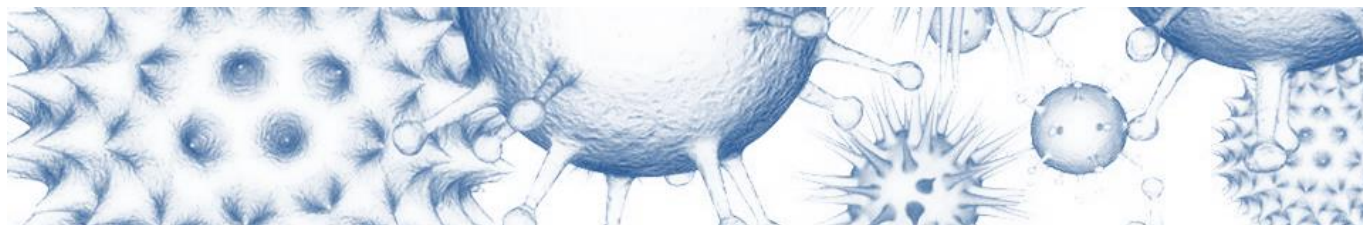
Microbiology and immunology Department, Georgetown University, Washington DC, USA

Primary woodchuck hepatocytes (PWHs) derived from animals with chronic WHV infection represent a useful liver cell culture model for various antiviral and immunotherapeutic applications.

PWH cultures were generated from the livers of chronic WHV carrier woodchucks and of WHV-naïve animals using the collagenase-perfusion method. The antiviral effects and associated changes in host innate immune response were investigated following treatment with lamivudine (3TC) and recombinant, woodchuck-specific interferon-alpha 5 (wIFN). Untreated PWHs served as a control. Cellular DNA and RNA were collected at various pre- and post-treatment time points. Aliquots of DNA and RNA then were used for determining changes in WHV replication while other RNA aliquots were analyzed for changes in host innate immune response. Selected immune genes included MX1, OAS, HLA-A, IRF7, IRF9, ISG15, MYD88, TAP1, TLR4, and TLR7, all of which have been implicated to play a role in IFN-mediated antiviral effects on HBV and hepatitis C virus.

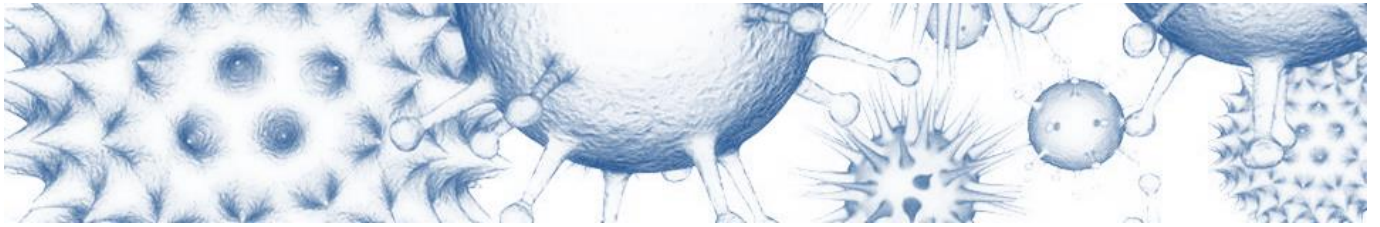
Treatment with 3TC for 5-7 days lowered the levels of WHV DNA and RNA by approximately 40% but reduced WHV replication was not associated with changes in innate immunity. The antiviral effects induced by wIFN were less pronounced after 5-7 days of treatment but marked changes in host immune response gene expression were observed. Peak and magnitude of expression differed between the tested genes allowing a preliminary characterization of genes that are likely involved in wIFN-mediated antiviral effects.

The data derived from treatment with a direct antiviral and an immunomodulator will be useful for the in vitro testing of novel drugs with unknown effects on WHV replication and innate immunity.

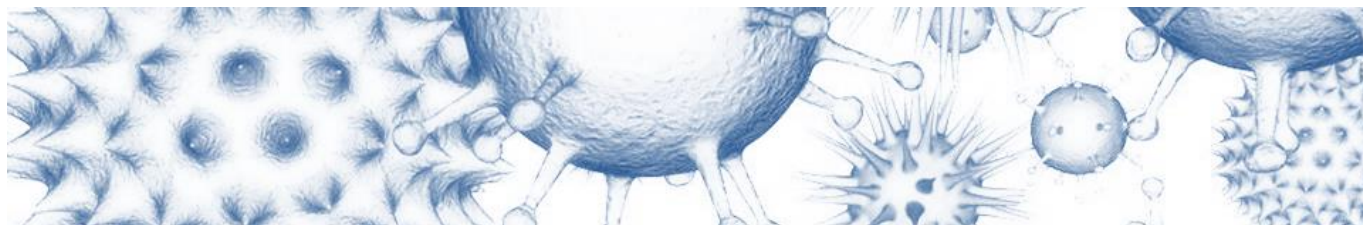


High-throughput screening for broad-spectrum chemical inhibitors of RNA viruses

Novel acyclic phosphonate nucleoside analogues with oxime fragment in the chain. Synthesis and antiviral activity. Aminooxy group as a convenient substrate for drug development



Thematic group discussion



Thematic group discussion: topics and program

Theme 1: Methods of screening

Discussion leaders:

Eric Freed, Mamuka Kvaratskhelia, Reuben Harris, Stuart F.J. Le Grice, Birgitta M. Wöhr

Theme2: Drug design

Discussion leaders:

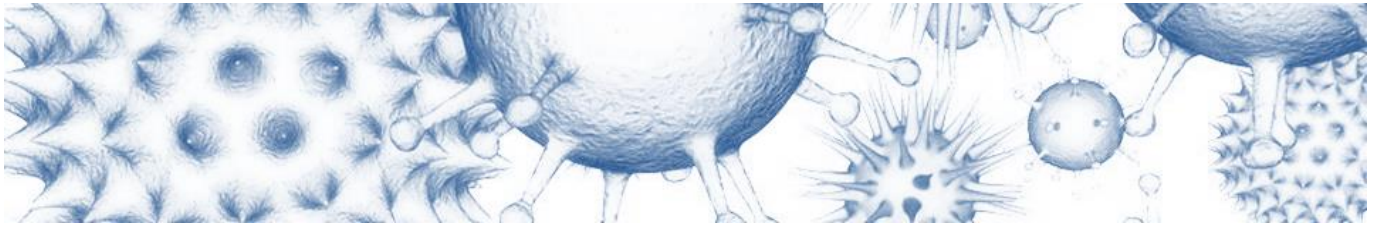
Maurizio Botta, Maria José Camarasa, Federico Gago, Elias Maccioni, Péter Mátyus, Roberto Di Santo

Theme3: Drug target

Discussion leaders:

Stephan Becker, Ben Berkhout, Thomas Mertens, Giorgio Palù, Cristina Parolin, Enzo Tramontano

	Methods of screening		Drug design		Drug target	
Discussion leaders	Le Grice Kvaratskhelia	Harris, Wöhr, Freed	Botta, Gago, Maccioni	Mátyus, Camarasa, Di Santo	Becker, Palù, Tramontano	Mertens, Berkhout, Parolin
Day1	Group 1	Group 2	Group 3	Group 4	Group 5	Group6
Day2	Group 3	Group 4	Group 5	Group6	Group 1	Group 2
Day3	Group 5	Group6	Group 1	Group 2	Group 3	Group 4



Groups

Group 1

Fatima Ezzahra AGHARBAOUI
Antonella ARRIDU
Jessica BOGS
Marta CADEDDU
Roberta CADONI
Michele CELESTINO

Group 2

Nicole GRANDI
Pouya HASSANDARVISH
Samira KHIAR
Florenca Natalia LINERO
Valentina Noemi MADIA

Group 3

Angela CORONA
Vasanthanathan POONGAVANAM
Udaya Pratap SINGH
St Patrick REID
Axel SCHUBERT,
Lora SIMEONOVA

Group 4

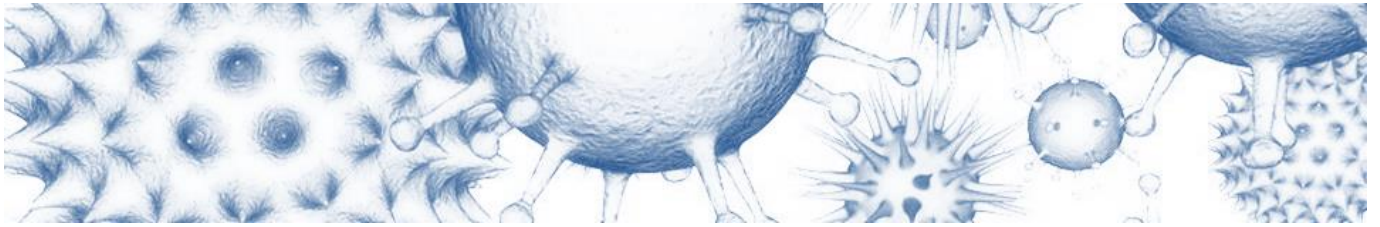
Valeria CANNAS
Ilaria CARLI
Laura CONTARDI
Simona DISTINTO
Janne TYNELL

Group 5

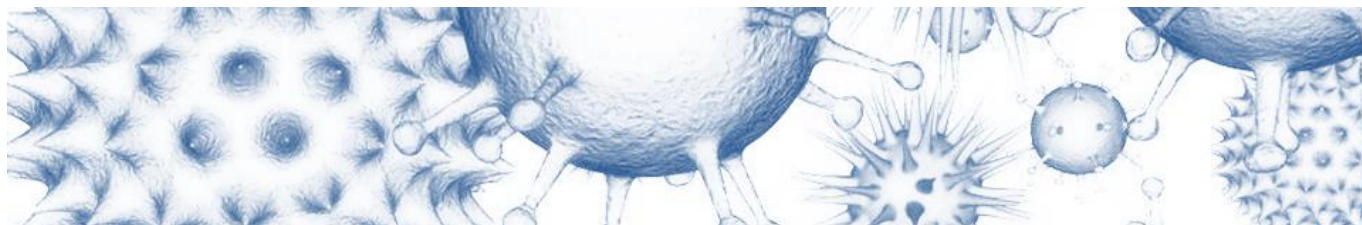
Riccardo MARTINI
Serena MASSARI
Elena MATYUGINA
Gianluca DAINO
Antonella MESSORE
Marta MURREDDU

Group 6

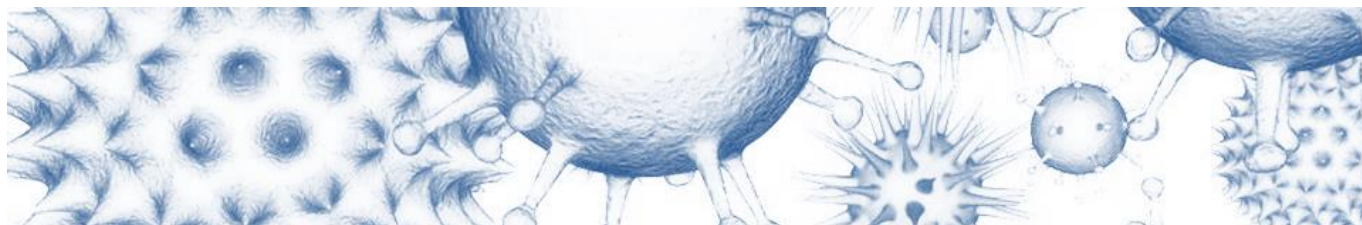
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