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UNIVERSITÀ DEGLI STUDI DI CAGLIARI

THIRD LIGHT RETREAT DiSVA

DiSVA

Monserrato, 7th February 2025

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7th FEBRUARY 2025

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Sezione di Biologia Animale ed Ecologia
Sezione Biomedica
Sezione di Botanica
Sezione di Neuroscienze ed Antropologia
Sezione di Scienze del Farmaco
Sezione di Scienze Farmaceutiche, Farmacologiche e Nutraceutiche

Slot	Event
08:00-08:40	Icebreaking coffee
Welcome and opening remarks	
08:40-09:00	Greetings from the authority and Director's Introductory Talk
New projects at DISVA	
09:00-09:30	New projects at Sezione di Biologia Animale ed Ecologia – Prof. Antonio Pusceddu
	New projects at Sezione Biomedica – Prof. Fabian Feiguin
	New projects at Sezione di Scienze Botaniche – Prof.ssa Cinzia Sanna
	New projects at Sezione di Neuroscienze ed Antropologia – Prof. Vito Sparacello
	New projects at Sezione di Scienze del Farmaco – Prof.ssa Chiara Sinico
	New projects at Sezione di Scienze Farmaceutiche, Farmacologiche e Nutraceutiche – Prof. Francesco Corrias
Session I - (Selected speakers for Sezione di Biologia Animale ed Ecologia)	
09:30-09:40	**Francesco Palmas –** *New approaches for assessing vulnerability of coastal lagoons in climate change*
09:40-09:50	**Andrea Bellodi –** *Beyond halieutic resource management: the MED.I.T.S Project as a persistent source of know-how and scientifc production*
09:50-10:00	**Alessandro Cau –** *Effect of microplastic contamination on ecosystem functioning*
Session II - (Selected speakers for Sezione di Biomedica)	
10:00-10:10	**Sante Scognamiglio** *– Comprehensive Characterization of RIG-I and IFN-α2A Impact on HERV Expression and Immune System Activation*
10:10-10:20	**Cristina Contini –** *Top-down proteomic approach to investigate cerebrospinal fluid from patients affected by Multiple Sclerosisand Neuromyelitis optica*
10:20-10:30	**Sonia Floris –** *In vitro and In vivo Hypoglycemic Effect of Ptilostemon casabonae and Rubus ulmifolius Extracts: A PromisinStrategy for Type 2 Diabetes Management*
Session III - (Selected speakers for Sezione di Botanica)	
10:30-10:40	**Maria Enrica Boi –** *An integrated approach of in situ and ex situ conservation: the Life Seedforce experience*
10:40-10:50	**Alba Cuena –** Ecological shifts over time in post-mining and quarry acquatic habitats
10:50-11:00	**Erika Bazzato –** *Vulnerability and resilience of Sardinina coastal ecosystems to climate change: a risk-based approach*
11:00-11:30	Coffee Break & poster viewing

Session IV - (Selected speakers for Sezione di Neuroscienze ed Antropologia)	
11:30-11:40	**Laura Dazzi –** *Role of the mesocortical dopaminergic system in mediating the cognitive symptoms of multiple sclerosis*
11:40-11:50	**Eduardo Pizzo Junior –** *Body composition as a tool for tailoring therapeutic intervetions in epilepsy*
Session V- (Selected speakers for Sezione di Scienze del Farmaco)	
11:50-12:00	**Matteo Perra –** *Enhanced control of iron overload via liposomes delivering antisense oligonucleotides*
12:00-12:10	**Alessia Onali –** *Broad-spectrum antivirals: unlocking defenses against viral threats*
12:10-12:20	**Mariano Andrea Scorciapino –** *Physicochemical investigations of transport processes across biological membrane models*
Session VI - (Selected speakers for Sezione di Scienze Farmaceutiche, Farmacologiche e Nutraceutiche)	
12:20-12:30	**Davide Moi –** *Investigation of cinnamic ester derivatives as antiviral compounds*
12:30-12:40	***Cristina Manis*** *- Lipidomics: when the intelligible becomes knowable*
12:40-12:50	***Alessandro Atzei*** *- Multiresidue methods analysis to detect contamination of selected metals in honey and pesticides in honey and pollen*
12.50-14:40	Light Brunch & poster viewing
Conclusions and closing remarks	
14:40-15:00	**Enzo Tramontano** Conclusions & Remarks

LIST OF PHD STUDENTS POSTERS

n°	Name	Surname	Title	Section
1	Eleonora	Metta	Seasonal virus-host dynamics in hypersaline environment: evidence supporting the Piggyback-the-Winner hypothesis	Biologia Animale ed Ecologia
2	Marta Maria	Cara	The expression of Human Endogenous Retroviruses in PBMC is modulated by SARS-CoV-2 acute infection and shows a specific transcriptional pattern as compared to other COVID-19 clinical stages	Biomedica
3	Marianna	Camasta	Characterization of the mechanisms of interferon production inhibition by Ebola virus VP35 wild-type and mutants	Biomedica
4	Sara	Piras	Differential expression of Human Endogenous Retroviruses in Chronic and Acute Myeloid Leukemia at Diagnosis and after TKI Therapy	Biomedica
5	Laura	Dettori	2-Phenylquinoline Derivatives Activity On SARS-CoV-2 Nsp13 Helicase: Insights From Enzymatic And Cell-Based Assays	Biomedica
6	Saili	Chabukswar	Reconstructing The Retroviral Envelope Ancestral Prototypes To Uncover Fusogenic Activity Of Human Endogenous Retroviruses	Biomedica
7	Claudia	Cabiddu	Identification of specific HERV loci differentially expressed in Multiple Sclerosis patients as potential biomarkers and therapeutic targets.	Biomedica
8	Roberta	Emmolo	Indolyl-DKA Derivatives as SARS-CoV-2 Nsp13 Inhibitors Blocking Coronaviruses Replication	Biomedica
9	Paolo	Malune	In-Silico Identification And Biochemical Validation of Novel SARS-CoV-2 RdRp Non-Nucleoside Inhibitors	Biomedica
10	Salvatore	Nieddu	Optimization of high-sensitivity assay for HTS of potential inhibitors against WNV NS2B-NS3 protease.	Biomedica
11	Sante	Scognamiglio	Comprehensive Characterization of RIG-I and IFN-2A Impact on HERV Expression and Immune System Activation	Biomedica
12	Silvia	Macis	The Impacts of Climate Change on Coastal Wetlands in Mediterranean Regions	Botanica

13	Stefano Francesco	Farci	Ecophysiology of Photosynthesis in Posidonia oceanica: A way to monitor climate change and fluctuations in the Mediterranean Sea	Botanica
14	Ludovica	DessÏ	Ecophysiology of seeds germination of plants with high conservation interest in the Mediterranean Basin	Botanica
15	Hicham	El Zein	Distribution patterns and conservation aspects of the vascular flora endemic to Lebanon	Botanica
16	Michele	Defraia	Are the targets of the Nature Restoration Law achievable at a local scale? An analysis of Natura 2000 sites on the island of Sardinia	Botanica
17	Benedetta	Gori	A comprehensive checklist of Mediterranean Wild Edible Plants	Botanica
18	Laura	Flore	Genetics and Athletic Performance: Speed and Strength in Young Footballers	Neuroscienze e Antropologia
19	Laura	Doro	Animal model of relapsing remitting multiple sclerosis (RRMS); differences between a long-term treatment and a short-term treatment	Neuroscienze e Antropologia
20	Federica	Frau	Decoding bioelectrical phase angle: a body fat indices perspective	Neuroscienze e Antropologia
21	Alessio	Pittiu	Production of liposomes by microfluidics: the impact of post-manufacturing dilution on drug encapsulation and lipid loss	Scienze del Farmaco
22	Valentina	Masala	The impact of solvent choice on the extraction of bioactive compounds from Cynara cardunculus (var. scolymus, sylvestris, and altilis) leaf by-products	Scienze del Farmaco
23	Erica	Sanna	Design, synthesis and biological evaluation of Pan-hCoV helicase inhibitors	Scienze del Farmaco
24	Giulia	Atzeni	Unveiling the potential of pyrazoles as West Nile NS3 helicase inhibitors	Scienze del Farmaco
25	Laura	Demuru	A combined computational and biochemical approach for the identification of ZIKV NS3pro potential allosteric inhibitors	Scienze del Farmaco

P1_Seasonal virus-host dynamics in hypersaline environment: evidence supporting the Piggyback-the-Winner hypothesis

E. Metta[1], F.L. Rohwer[2], E. Tramontano[1], A. Pusceddu[1], N. Grandi[1]

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Prokaryotes are the most abundant cellular organisms on Earth and serve as primary hosts for viruses. Microbial and viral communities play pivotal roles in marine food webs. Viral-induced cell lysis prevents species dominance, as explained by the "Kill-the-Winner" hypothesis. This model suggests high microbial density correlates with high viral density, an elevated high virus-to-microbe ratio (VMR), and increased lytic infections. However, recent findings support the "Piggyback-the-Winner" (PtW) hypothesis, which proposes that high microbial density reduces VMR due to increased lysogeny.
This study investigates virus-host interactions in salterns to assess whether higher host density corresponds to lower viral density and a shift from lytic to temperate dynamics.
Water samples were collected in May and August 2024 from "Contivecchi" saltworks across four ponds with varying salinity. Microbial and viral abundances were quantified via epifluorescence microscopy. Viral particles were concentrated using PEG precipitation and CsCl ultracentrifugation, with recovery efficiency assessed via epifluorescence microscopy. Viral DNA was extracted using formamide lysis, CTAB, and phenol-chloroform, then sequenced.
Data revealed seasonal variations in microbial and viral abundance. In May, VMR ranged from 6.4 to 26.6, with higher values in high-salinity ponds. In August, VMR decreased across all ponds, suggesting a potential shift to lysogeny infections. These findings align with the PtW hypothesis, where lytic dynamics are supressed at high host density due to increased lysogeny. Virome analyses will further elucidate microbial density's impact on viral communities, particularly changes in viral functional diversity, which may decline with rising microbial density - an indicator of temperate viral communities.

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

Nicole Grandi[1], Marta Maria Cara[1], Jessica Milia[2], Roberto Cusano[2], Maria Itria Monne[3], Giovanna Piras[3], Maura Fiamma[3], Angelo Domenico Palmas[3], Enzo Tramontano[1]

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The modulation of Human Endogenous Retroviruses (HERV) is able to sustain innate immune activation in various infectious and inflammatory contexts due to the expression of immunogenic viral transcripts and, eventually, immunogenic proteins[1]. SARS-CoV-2 infection is known to stimulate an important inflammatory response, which characterizes COVID-19 pathogenesis.
To give an understanding on this poorly defined interplay, we performed the high-throughput sequencing and differential expression analysis of ~3300 HERV loci in the peripheral blood mononuclear cells (PBMC) of 37 individuals with acute SARS-CoV-2 infection and 8 healthy controls (HC). PBMC have been chosen as these cells are not directly infected by the virus but have a crucial role in the inflammatory and immune events defining the COVID-19 pathogenesis.

Results showed that SARS-CoV-2 infection modulates HERV expression and allows to clearly divide infected individuals from HC in unsupervised clustering analyses. Differential expression analyses confirmed that a total of 359 HERV loci were significantly modulated in the presence of SARS-CoV-2 infection and 253 of them were upregulated.
Finally, the obtained transcriptional signature during SARS-CoV-2 acute infection has been compared with previous results obtained in the PBMC from convalescent and retesting positive patients, revealing a specific pattern of HERV modulation but also a subset of HERV loci significantly modulated in all COVID-19 clinical stages[3].
The present study shows a comprehensive picture of the HERV transcriptome in PBMC and its modulation in HC as compared to different COVID-19 clinical stages, thought to be relevant to the disease clinical manifestation and outcome.

P.3_Characterization of the mechanisms of interferon production inhibition by Ebola virus VP35 wild-type and mutants

Marianna Camasta[1], Elisa Fanunza[1], Luca Zinzula[2], Enzo Tramontano[1]

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

P.4_Differential expression of Human Endogenous Retroviruses in Chronic and Acute Myeloid Leukemia at Diagnosis and after TKI Therapy

Sara Piras[1], Maria Monne[2], Giovanna Piras[2], Roberto Cusano, Antonella Uras[2], Marco Murineddu[2], Alessandro Murgia[2], Angelo D. Palmas[2], Enzo Tramontano[1] and Nicole Grandi[1]

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Human Endogenous Retroviruses (HERVs) represent the ~8% of the human genome. They are involved in physiological functions and able to both modulate and be influenced by the host immune system. Despite this could likely led to pathological manifestation, especially in cancer where HERV

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

P.5_2-Phenylquinoline Derivatives Activity On SARS-CoV-2 Nsp13 Helicase: Insights From Enzymatic And Cell-Based Assays

Laura Dettori[1,2], Roberta Emmolo[1], Giada Cernicchi[3], Maria Giulia Nizi[3], Angela Corona[1], Oriana Tabarrini[2], Enzo Tramontano[1]

[1]University of Cagliari, Italy; [2]National PhD Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, 27100, Italy; [3]University of Perugia, Italy

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative agent of the Coronavirus Disease 19 (COVID-19) pandemic, leading to significant global morbidity and mortality. SARS-CoV-2 relies on a complex machinery for the replication and transcription of its genome. A crucial component of this machinery is the non-structural protein 13 (nsp13), which presents a helicase activity. With multiple enzymatic functions, including unwinding and nucleoside phosphatase (NTPase) activities.
Given its pivotal role in viral replication, nsp13 is a promising target for antiviral drug development. The inhibition of nsp13 helicase activity can disrupt the replication cycle of SARS-CoV-2 potentially leading to reduced viral load and amelioration of disease severity.
In this study, we performed ATPase and unwinding assays to evaluate the activity of some 2-phenylquinoline derivatives designed and synthesized based on a previous hit, on SARS-CoV-2 nsp13. Results showed that some of them were able to inhibit both the nsp13 unwinding and NTPase activities with IC_{50} values in the micromolar range. Compounds were also tested on viral replication in SARS-CoV-2-infected VeroE6 cells. A few of them showed an inhibitory activity in the micromolar range and no cytotoxicity (CC_{50}> 100 mM).

P.6_ Reconstructing The Retroviral Envelope Ancestral Prototypes To Uncover Fusogenic Activity Of Human Endogenous Retroviruses

Saili Chabukswar[1], Nicole Grandi[1], Enzo Tramontano[1]
[1]Laboratory of Molecular Virology, Department of Life and Environmental Sciences, University of Cagliari, 09042 Cagliari, Italy

Human Endogenous Retroviruses (HERVs) are the remnants of the ancient viral infections that are accumulated in the genome and constitute approximately 8% of the genome during evolution. A general proviral structure is composed of *gag, pro, pol* and *env* genes flanked by long terminal repeats (LTRs) at both 5' and 3' ends. The envelope (Env) proteins of HERVs are known for their fusogenic properties the cellular receptors that mediate these interactions remain largely unidentified. This is because most of the HERV sequences have eventually become inactive due to either the disruption of the open reading frames (ORFs) by mutations or completely losing the viral genes by recombination through various factors. The study aimed to reconstruct representative consensus sequences for envelope (Env) proteins across diverse human ERV (HERV) groups and further explores the molecular interactions between reconstructed HERV envelope (Env) proteins and host cell receptors. By applying a comprehensive bioinformatics pipeline we reconstructed Env proteins for 32 HERV groups across Class I (gamma- and epsilon-like), Class II (beta-like), and Class III (spuma-like) members. The reconstructed sequences were curated to preserve functional domains and proper reading frames. Phylogenetic analyses revealed class-specific clustering with exogenous retroviruses, confirming the accuracy of reconstructed Env prototypes. Out of 32 reconstructed HERV Env proteins, we focused on experimentally verifying the fusogenic activity of HERV-W, HERV-T, HML2-Rec, and HML-2 Np9. We observed that the reconstructed HERV-W and HERV-T exhibited syncytia formation at specific time points while HML2-Rec and HML2-Np9 displayed limited fusion activity under same conditions. Overall, by combining bioinformatics and experimental approaches we aim to better understand the fusogenic activity of the HERVs with the host cellular receptors.

P.7_ Identification of specific HERV loci differentially expressed in Multiple Sclerosis patients as potential biomarkers and therapeutic targets.

Claudia Cabiddu[1], Alessia Caredda[1], Jessica Frau[2], Eleonora Cocco[2], Nicole Grandi[1] and Enzo Tramontano[1]

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

P.8_ Indolyl-DKA derivatives as SARS-CoV-2 nsp13 inhibitors blocking coronaviruses replication

R. Emmolo [1], VN. Madia [2], S. Maloccu [1], L. Dettori [1], G. Ruggieri [2], A. Albano [2], R. Di Santo [2], R. Costi [1],[2]E. Tramontano [1] and A. Corona[1]

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The outbreak of the novel coronavirus SARS-CoV-2 in late 2019 has presented an unprecedented global health challenge and highlighted the risk of a zoonotic spillover into human population, bringing the attention on the development of coronaviruses antivirals.
For RNA viruses, RNA helicases have been recognized to play critical role in viral replication cycles and showed a high sequence identity among all known coronaviruses. Considering that SARS-CoV-2 helicase (nsp13) lacks homologous proteins in humans and other mammals, it could be a good target for discovering selective antiviral inhibitors.
Nsp13 is a multidomain enzyme able to unwind DNA or RNA in an NTP-dependent manner with a 5'-3' polarity. It couples two C-terminal RecA ATPase domains, characteristic of the 1B (SF1B) helicase superfamily, with other three domains: the N-terminal zinc-binding domain (ZBD), essential for the helicase activity, a stalk, and a 1B domain.
In the present study, we exploit a new class of indolyldiketoacid derivatives recently identified as nsp13 inhibitors, starting from the optimization of our previous diketoacid (DKA) hits exhibiting broad-spectrum antiviral activity.
The new compounds were tested on both the SARS-CoV-2 nsp13 unwinding and ATPase associated activities. Many of them showed the capacity to inhibit both nsp13 enzymatic functions in a significant low micromolar range and were selected for the evaluation of their activity in blocking SARS-CoV-2 replication. Moreover, these indolyl DKAs provided a strong rationale to evaluate their potential broad-spectrum antiviral activity, testing their capacity to block viral replication of other coronaviruses such as HCoV229E.

P.9_ In-Silico Identification And Biochemical Validation of Novel SARS-CoV-2 RdRp Non-Nucleoside Inhibitors

Paolo Malune[1], Daniela Iaconis[2], Candida Manelfi[2], Roberta Emmolo[1], Stefano Giunta[1], Annalaura Paulis[1], Valentina Piras[1], Andrea Beccari[2],Angela Corona[1], Enzo Tramontano[1], Francesca Esposito[1]

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Since its discovery in 2019, SARS-CoV-2 has continued to spread globally, resulting in over 7 million deaths worldwide [1]. Among the viral non-structural proteins, nsp12 functions as the viral RNA-dependent RNA polymerase (RdRp), along with its cofactors nsp7 and nsp8[2]. To date, only two drugs targeting viral enzymes, Remdesivir and Paxlovid, have been authorized by the EMA for the treatment of COVID-19[3]. This study aims to identify and validate new SARS-CoV-2 inhibitors through in silico, biochemical, and cell-based screenings. In collaboration with Dompé farmaceutici, two libraries of safe-in-man and natural compounds were screened in silico against the SARS-CoV-2 nsp12/7/8 complex using the Exscalate platform, targeting the orthosteric and two allosteric sites of nsp12. Results were filtered based on docking scores, novelty of the target (according to

literature), and known safety from clinical trials, and then classified by docking site number and type. Following hit selection process, 119 compounds were identified and subsequently screened in a biochemical assay to test their ability to inhibit SARS-CoV-2 nsp12 polymerase activity in the presence of nsp7 and nsp8. The four most active hits on the RdRp enzymatic activity were then tested against SARS-CoV-2 replication on Vero E6 cells in a primary screening, displaying a promising antiviral activity.

[1] https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
[2] Steiner, S., Kratzel, A., Barut, G. T., Lang, R. M., Aguiar Moreira, E., Thomann, L., Kelly, J. N., & Thiel, V. (2024). SARS-CoV-2 biology and host interactions. Nature reviews. Microbiology, 22(4), 206–225. https://doi.org/10.1038/s41579-023-01003-z
[3] https://www.aifa.gov.it/aggiornamento-sui-farmaci-utilizzabili-per-il-trattamento-della-malattia-covid19

P.10_Optimization of high-sensitivity assay for HTS of potential inhibitors against WNV NS2B-NS3 protease.

S. Nieddu[1], P. Malune[1], E. Tramontano[1] and F. Esposito[1]
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West Nile virus (WNV) is a human pathogen belonging to the *Flaviviridae* family, responsible of widespread disease for which there is no vaccine currently available [1]. One attractive target for antiviral development is the viral trypsin-like serine protease NS2B-NS3, a heterodimeric complex between the hydrophilic domain of the cofactor, NS2B (NS2BH) and the protease domain (NS3-pro) [2]. During viral replication, the NS2B-NS3 protease, together with host proteases is responsible for the cleavage of the flavivirus polyproteins, leading to active viral proteins [3]. Despite strong efforts in the development of antivirals due to its essential role in the viral replication, no protease inhibitors have reached clinical trials yet [4]. We expressed and purified the viral protease, obtaining a high-purity product and optimized a FRET-based fluorescent assays, employing a labelled peptide substrate. We determined the optimal assay conditions, such as reaction mix composition, enzyme and substrate concentration and optimal incubation time, obtaining an assay with high sensitivity and specificity, useful for the enzymatic characterization and high-throughput quantitative screening (HTS) of potential inhibitors. The optimized assay proved to be a rapid, reproducible tool with a robust Z factor, for screening potential inhibitors against the WNV NS2B-NS3 viral protease. The validity of the assay was demonstrated by confirming the inhibitory activity of aprotinin, used as a positive control given its known ability to inhibit the WNV NS2B-NS3 protease. Screening of compounds given by our collaborators is on-going.

[1] S. Ulbert, «West Nile virus vaccines – current situation and future directions», *Hum. Vaccines Immunother.*, vol. 15, fasc. 10, pp. 2337–2342, ott. 2019, doi: 10.1080/21645515.2019.1621149.
[2] N. H. Mueller, C. Yon, V. K. Ganesh, e R. Padmanabhan, «Characterization of the West Nile virus protease substrate specificity and inhibitors», *Int. J. Biochem. Cell Biol.*, vol. 39, fasc. 3, pp. 606–614, 2007, doi: 10.1016/j.biocel.2006.10.025.
[3] S. K. Samrat, J. Xu, Z. Li, J. Zhou, e H. Li, «Antiviral Agents against Flavivirus Protease: Prospect and Future Direction», *Pathogens*, vol. 11, fasc. 3, p. 293, feb. 2022, doi: 10.3390/pathogens11030293.
[4] S. Voss e C. Nitsche, «Targeting the protease of West Nile virus», *RSC Med. Chem.*, vol. 12, fasc. 8, pp. 1262–1272, 2021, doi: 10.1039/D1MD00080B.

P.11_Comprehensive Characterization of RIG-I and IFN-α2A Impact on HERV Expression and Immune System Activation

Sante Scognamiglio[1], Nicole Grandi[1], Luciano Cascione[2,3], Francesco Bertoni[2,4], Angela Corona[1], Enzo Tramontano[1]

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[2] Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Bellinzona, Switzerland
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Human Endogenous Retroviruses (HERVs) constitute 8% of our genome and are currently highly investigated for their possible contribution to human physiology and pathology. Their integration and fixation have shaped the genome over time, with HERV proviruses and solitary LTRs playing key roles in primate genome evolution, particularly through their intricate interactions with the immune system. We analysed HERV transcriptional modulation following immune system activation, in a human non-tumoral cell line (BEAS-2B) at two distinct checkpoints: RIG-I, a key pattern recognition receptor (PRR) for RNA virus detection, and the downstream interferon receptors (IFNAR). For RIG-I activation we stimulated with 3p-hpRNA, a specific RIG-I agonist, and for IFNAR activation we stimulated with IFN-α2A, which activates the interferon cascade downstream. Our results demonstrated that immune stimulation significantly influenced HERV expression, with 72 differentially expressed HERVs (deHERVs) out of 3284 HERV loci following RIG-I activation as compared to only 3 loci under IFN-α2A, highlighting RIG-I's broader impact due to its upstream role in immune pathways. The observed co-localization of deHERVs with modulated immune-related genes, such as CMPK2 and IFIT family members, pointed out their strong transcriptional relationship and the possible role of HERVs in the physiological expression of these genes. Furthermore, through a machine learning approach we identified immune and tumor-associated genes likely involved in regulating HERV loci expression, with regulatory statuses correlated with the direction of HERV expression. These findings underscore the intricate interplay between HERVs and immune activation, suggesting a broader modulation of biological processes beyond classical immune response.

P.12_The Impacts of Climate Change on Coastal Wetlands in Mediterranean Regions

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Climate change increasingly threatens Mediterranean climate regions, particularly coastal wetlands, whose vulnerability is well documented but under researched. Specific studies on the ecological dynamics of these ecosystems remain limited. To address this gap, we conducted a systematic review of literature from Scopus and Web of Science (1992–2024), categorizing studies by climate change factors, environmental impacts, methodologies, and geographic scale. Of 826 articles initially screened, 70 specifically examined climate change effects on Mediterranean coastal wetlands, with 96.6% focusing on the Mediterranean Basin. Key factors identified included sea level rise (SLR), salinity, increased temperatures, extreme events, altered precipitation, and drought. SLR emerged as the most studied factor, whose impact affects generalized wetland systems, soil, vegetation, and coastal human heritage. Secondly, the effects of salinity were highlighted as significantly influencing

plant and animal communities, disrupting freshwater habitats, and degrading soil health. Rising temperatures were mainly correlated with the detriment of biological and physical processes, particularly vegetation and wildlife, while extreme events were reported as factors exacerbating damage across ecosystems. Conversely, precipitation and drought received comparatively less attention. Methodologically, most studies employed modelling, IPCC scenarios, and experimental work at local and regional scales, and none at the global level. Effective conservation requires identifying vulnerable species and understanding adaptations. Expanding research to underrepresented Mediterranean climate regions globally would provide valuable insights into shared vulnerabilities and adaptive strategies, fostering a comprehensive approach to mitigating climate change impacts on coastal wetlands**.**

P.13_ Ecophysiology of Photosynthesis in Posidonia oceanica: A way to monitor climate change and fluctuations in the Mediterranean Sea

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Posidonia oceanica represents an important case of study for the ecophysiology of photosynthesis. This species is equipped with an extremely versatile photosynthetic apparatus that is able to proliferate at different bathymetries, thus under limiting conditions of light quality and quantity. Light composition relative to bathymetry has played a fundamental role in the evolution of the photosynthetic apparatus in this species. The light spectrum is substantially influenced by bathymetry, such that at a depth of 20 m, about 50% of the blue component and 100% of the red component are lost, respectively. This means that the functional and structural organization of the photosynthetic apparatus must adapt its antenna system, particularly the Light Harvesting Complexes I and II (LHCI and LHCII), as well as the balance between Photosystem I and II (PSI and PSII). The aim of this study is to understand how light quality and quantity have affected the organization of the photosynthetic apparatus and to study the plasticity of the apparatus in response to changes in light quality and quantity with respect to bathymetry.

P.14_ Ecophysiology of seeds germination of plants with high conservation interest in the Mediterranean Basin

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The Mediterranean Basin is a biodiversity hotspot. Sardinia, the second-largest Mediterranean island, hosts over 3,200 *taxa*, 13-15% of which are endemic. Many of these species are in unfavorable conservation status, with 16% listed as Critically Endangered (CR) or Endangered (EN) by the IUCN Red List.
This project investigated the germination ecophysiology of five Sardinian endemic species (*Astragalus maritimus*, *A. verrucosus*, *Limonium strictissimum*, *Linum mulleri*, and *Linaria flava* subsp. *sardoa*) which are included in Annexes II and IV of the Habitats Directive 92/43/EEC. The research was conducted as part of the LIFE SEEDFORCE project.

Studies on the two *Astragalus* species revealed that mechanical scarification effectively breaks physical dormancy, whereas fire-induced treatments do not. *Limonium strictissimum* demonstrated high germination capacity and recovery potential after salt stress, highlighting its adaptation to coastal environments. Germination of *Linum mulleri* seeds occurred principally at 15–20°C, with gibberellic acid (GA_3), warm stratification (W) and dry after ripened (DAR) treatments expanding the thermal range and overcoming physiological dormancy. *Linaria flava* subsp. *sardoa* exhibited inter-population variability, with GA_3 promoting germination, suggesting the presence of physiological dormancy. The analysis of *in situ* soil temperature recorded by data loggers suggested that prolonged high temperatures might be necessary for dormancy release.
This research highlights the variability in germination strategies among Mediterranean endemic species and the importance of understanding germination mechanisms for conservation purposes. The findings provide critical insights for developing *ex situ* protocols and optimizing *in situ* conservation efforts, including translocation programs, to ensure the survival of these threatened species.

P.15_ Distribution patterns and conservation aspects of the vascular flora endemic to Lebanon

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Lebanon is a biodiversity hotspot in the Mediterranean, home to unique flora, including endemic vascular species. However, more data is needed on their distribution and ecological needs. Identifying priority areas for plant diversity is crucial for effective conservation. This study has several components: the first updates the list of vascular plant species endemic to Lebanon. These species are important for research and conservation as they represent the distinctive floral heritage of the country. The second investigates the relationship between endemic species distribution and environmental factors, aiming to define biogeographical units within Lebanon. The third chapter focuses on environmental factors influencing pattern of endemic species richness, helping to identify micro- and nanohotspots of diversity. Chapters four and five characterize the ecology of plant communities rich in endemics and assess their distribution and conservation status. After reviewing literature and examining around 1,600 specimens from the herbaria of Beirut, Paris, Geneva, Kew, and Edinburgh, a total of 173 endemic species from Mount Lebanon, Anti-Lebanon, and Mount Hermon were listed. Approximately 50% of these species are assessed as Vulnerable (15), Endangered (50) or Critically Endangered (14) according to IUCN guidelines and criteria. Field data collected from April to September 2024, across 700 plots from the coast to summits at 3,000 m, yielded approximately 8,000 observations of native plant species, providing an initial overview of the most important areas for floral diversity.

P.16_ Are the targets of the Nature Restoration Law achievable at a local scale? An analysis of Natura 2000 sites on the island of Sardinia

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The Nature Restoration Law (NRL) aims to restore 20% of degraded terrestrial and marine ecosystems across Europe by 2030. One of the initial provisions states that, by 2030, Member States should prioritize the restoration of natural ecosystems within Natura 2000 sites, emphasizing the urgency of assessing the conservation status of habitats in these areas.
We selected Sardinia as a case study to evaluate the feasibility of the NRL at the local level. The Natura 2000 sites in Sardinia cover a comparable percentage of territory (18.87%) to the national (19.38%) and European level (18.6%). Additionally, Sardinia's insularity, high biodiversity levels, and low population density make it an ideal model for testing restoration strategies.
Using official Natura 2000 data provided by the Italian Ministry of the Environment, we assessed the potential for restoration for each habitat within each site based on the conservation status values.
The results indicated that coastal ecosystems were the most endangered. However, their limited distribution meant that their restoration would have a modest impact on achieving the NRL target. In contrast, forest and shrub habitats, which were more widely distributed, emerged as the main contributors to the restoration goals.
Conducting this study at a local level allowed us to provide actionable recommendations for management practices to be adopted in the region. Our findings confirmed that restoration efforts confined to Natura 2000 sites alone would be insufficient to meet the NRL targets, underscoring the need to implement additional restoration measures in agricultural, urban, and other natural and semi-natural areas.

P.17_ A comprehensive checklist of Mediterranean Wild Edible Plants

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Wild Edible Plants (WEPs) are essential components to Mediterranean food systems, yet knowledge gaps persist in their taxonomy, distribution, and culinary uses. Societal changes have led to a rapid decline in WEP consumption and erosion of the related Traditional Ecological Knowledge (TEK), threatening the integrity of the Mediterranean diet and hindering conservation efforts. Our study aims to address these challenges by retrieving, maximizing, and consolidating accessible knowledge on WEPs to enhance nature's contribution to people's lives in the Mediterranean Basin.

We present the first comprehensive catalogue of Mediterranean WEPs, integrating international datasets and extensive literature. This checklist includes accepted names for 2,716 taxa, related geographic and detailed ethnobotanical insights into their phytoalimurgic use across Mediterranean countries, revealing that 45% are absent from global edible plant databases. Additionally, 48% have documented medicinal uses, underscoring their nutraceutical potential. The richest families are Asteraceae (481 taxa), Lamiaceae (282), Fabaceae (226), and Apiaceae (184), with key genera including *Allium* (80), *Vicia* (53), *Thymus* (49), and *Salvia* (39). The most versatile species—*Foeniculum vulgare, Taraxacum* sp. pl., *Urtica dioica,* and *Portulaca* gr. *oleracea*—are also the most frequently cited. Hemicryptophytes (37.1%) dominate Raunkiær's life form categories, followed by therophyte (23.8%) and phanerophyte (15.6%). Most frequently used plant parts are leaves (1127), and most common food preparations belong to the category of "savoury preparations", and to the "vegetable dishes" and "egg dishes" subcategories, according to the Economic Botany Data Collection Standard (EBDCS).

By fostering data accessibility, we aim to empower communities and stakeholders to actively participate in the preservation of WEPs and related TEK, untapping the potential of extraordinary resources that can support the development of more diverse and sustainable food systems.

P.18_ Genetics and Athletic Performance: Speed and Strength in Young Footballers

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Genetic factors play a major role in athletic performance, contributing to approximately 66% of the variation in athletic ability (1). Specific genetic variants in the ACE, ACTN3, and MCT1 genes are linked to strength, speed, and endurance. The ACE gene affects muscle metabolism: the I allele is associated with endurance, while the D allele is linked to strength and sprinting (2,3). The ACTN3 gene, related to explosive force generation, is strongly connected to sprint performance, with the RR genotype showing better power performance (4). The MCT1 gene influences endurance by regulating lactate transport (5). This study examines the effects of ACE I/D, ACTN3 R577X, and MCT1 polymorphisms on muscle mass and athletic performance in young football players. DNA was collected via buccal swabs, and genotyping was done using PCR and RFLP-PCR. Results showed that ACTN3 polymorphism significantly impacted sprint performance, with RR players achieving faster 10m and 20m sprint times.

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2. Zhang et al., The I allele of the angiotensin-converting enzyme gene is associated with an increased percentage of slow-twitch type I fibers in human skeletal muscle, 2003, Clin Genet, 63(2):139-144.
3. Thompson et al., Association of genetic factors with selected measures of physical performance, 2006, Phys Ther, 86(4):585-591.
4. Pimenta et al., Effect of ACTN3 gene on strength and endurance in soccer players, 2013, J Strength Cond Res, 27(12):3286-392.
5. Fedotovskaya et al., A common polymorphism of the MCT1 gene and athletic performance, 2014, Int J Sports Physiol Perform, 9(1):173-180.

P.19_Animal model of relapsing remitting multiple sclerosis (RRMS); differences between a long- term treatment and a short-term treatment

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Multiple sclerosis (MS) is a chronic autoimmune disease that affects the central nervous system (CNS) leading to neurodegeneration, inflammation and demyelination (Compston & Coles, 2008). The abnormally activated immune system attacks myelin causing the reduction or interruption of nerve transmission (Dendrou et al., 2015). Myelinic damage, present in different areas of the CNS, causes various symptoms, such as motor, cognitive and sensory deficits (Dedoni et al., 2023; Jakimovski et al.2024).
The focus of scientific research has been on motor deficits rather than cognitive ones, which has led us to fill this gap. For our study we administered cuprizone (N,N1-bis (cyclohexanone) oxaldihydrazone) to rats as this represents the animal model used to mimic the most common form of the pathology, therelapsing remitting multiple sclerosis, (Dedoni et al.2023). Cuprizone is a copper chelating agent, which induces apoptosis of oligodendrocytes and activation of microglia and astrocytes, leading to demyelination of different areas, such as the corpus callosum, cerebral cortex and cerebellum (Basoglu,2013; Oakden et al., 2017; Silvestroff et al., 2012). It has been observed that discontinuation of cuprizone treatment after 5-6 weeks allows partial or complete remyelination, whereas exposure beyond 12 weeks induces a irreversible loss of myelin (Dedoni et al., 2023). As recently demonstrated by experiments done in our laboratory, long-term treatment with Cuprizone (six weeks) can significantly reduce cognitive performance, dependent on a reduction in the extracellular concentration of dopamine in the prefrontal cortex and a significant demyelination measured in the Corpus Callosum. We therefore decided to evaluate whether a shorter treatment (three weeks) followed by a washout period (three weeks) was able to repristinate the deficits in cognitive performance and in the basal concentration of dopamine in the prefrontal cortex. In this context, a behavioral task, the T-maze test, was carried out to analyze: i) forced alternation and ii) delayed not matched to position both as an index of working memory ; iii) reversal learning as an index of behavioural flexibility, dependent on the prefrontal cortex.
The results of the T-maze show that the animals exposed to the long term treatment with cuprizone have deficits in all three forms of memory investigated, which correlate to a decreased extracellurar concentration of dopamine in the prefrontal cortex. On the contrary, animals exposed to the short term treatment followed by a wash out period did not significantly differ from the control group on the tasks; in this group the extracellular concentration od dopamine in the prefrontal cortex also did notsignificantly differ from control group. These results confirm the initial thesis that, in the experimental model of RRSM, the removal of cuprizone from the diet followed by a wash out period may reduce the negative effects of demyelination induced by cuprizone, demonstrating a recovery of cognitive abilities. They also support the hypothesis that cognitive deficits are correlated with a decreased activity of mesocortical dopaminergic neurons. In conclusion, continuing research in this field is crucial to better understand the dynamics of MS and develop more effective treatments.

P.20_ Decoding bioelectrical phase angle: a body fat indices perspective

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Introduction: Phase angle (PhA) is an index derived from bioelectrical impedance analysis (BIA). It is associated with body cell mass, cell membrane integrity, skeletal muscle mass and body fluid distribution. The relationship with body fat is less clear, with inconsistent results between studies.
Aim: The aim of this study is to investigate the relationship of PhA with body mass index (BMI) and fat mass (FM and FM%, estimated using a gold standard technique) in a large sample of US young adults.
Methods: A cross-sectional sample of 1533 adults of both sexes from NHANES 2003-2004 was analysed. Selected variables were age, sex, BMI, PhA, FM and FM%. The relationship between PhA and body fat indices was examined using non-linear cubic spline regression models. Results were visualised using estimated functions and statistical significance was assessed using the F-test.
Results: In all models, the relationship between PhA and body fat indices is curvilinear, with slightly different patterns for each index, and is statistically significant ($p < 0.005$). The explained variance is limited (< 10%). PhA is positively associated with BMI and FM at lower body fat levels and negatively associated with all indices at higher body fat levels.
Conclusions: These results highlight a non-linear relationship between PhA and body fat. The decrease in PhA at high body fat levels suggests a loss of cellular integrity and altered body fluid distribution in obese patients.

P.21_ Production of liposomes by microfluidics: the impact of post-manufacturing dilution on drug encapsulation and lipid loss

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Microfluidic mixing is recognized as a convenient method to produce liposomes for its scalability and reproducibility. Numerous studies have described the effect of process parameters such as flow rate ratio (FRR) and total flow rate (TFR) on size and size distribution of vesicles [1,2]. In this work, we focused our attention on the effect of FRR on the encapsulation efficiency of liposomes, as we hypothesized that different amount of residual organic solvent can affect the retention of lipophilic drug molecules within the bilayer. In a further step, we investigated how the liposomes integrity and drug loading were impacted by a post-manufacturing dilution, comparing this process to a direct dialysis. Molecular dynamics simulations were also performed to better understand lipids and drugs behavior in the ethanol-water environment.
Our results highlight the need to tailor the purification method based on the lipophilic character of the loaded molecule to ensure high encapsulation and limit the waste of material. Molecular dynamics simulations evidenced that increasing ethanol concentration (i.e. higher FRRs) promotes greater dispersion of the drug. In contrast, at lower ethanol concentrations, the drug tends to form

clusters, which may impede leakage due to their larger size. Additionally, in the presence of lipids, the drug tended to co-segregate with them.

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[2] N. Forbes et al. *Int. J. Pharm.* **2019**, *556*, 68.

P.22_ The impact of solvent choice on the extraction of bioactive compounds from *Cynara cardunculus* (var. *scolymus*, *sylvestris,* and *altilis*) leaf by-products

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Two different ethanol/water ratios (EtOH:H_2O 20:80 and EtOH:H_2O 80:20) were used to extract leaf by-products from *C. cardunculus* var. *scolymus*, *sylvestris,* and *altilis*. The (poly)phenol content of the extracts was quali-quantitatively assessed using Folin-Ciocalteu's assay, (HR) LC-ESI-QTOF MS/MS, and LC-PDA [1]. The total amounts of bioactive compounds dosed by Folin-Ciocalteu's assay and LC-PDA revealed that, in terms of quantitative differences based on the EtOH:H_2O ratio used as the extraction solvent, EtOH:H_2O 80:20 extracts are consistently the richest in the total amount of dosed compounds when compared to EtOH:H_2O 20:80 extracts. Further differences are connected with the three varieties. Indeed, *C. cardunculus* var. *sylvestris* and var. *altilis* were very similar and had higher phenol content than *C. cardunculus* var. *scolymus*. Regarding the relative amount of the different classes of compounds, it can be noticed that the quantity of three main classes (hydroxycinnamic acids, flavonoids and others) varied according to the EtOH:H_2O ratio and the *C. cardunculus* variety. For instance, 43% of the compounds dosed in *C. cardunculus* var. *altilis* EtOH:H_2O 80:20 were flavonoids, while 51% and 59% of the compounds dosed in *C. cardunculus* var. *scolymus* and *C. cardunculus* var. *sylvestris*, both with EtOH:H_2O 80:20, were hydroxycinnamic acids. The results indicate that differences in the EtOH:H_2O ratio and *C. cardunculus* varieties can affect the total and relative amounts of the dosed bioactive compounds.
[1] Masala et al. Chemical profiling and evaluation of antioxidant activity of artichoke (*Cynara cardunculus* var. *scolymus*) leaf by-products' extracts obtained with green extraction techniques. Molecules, 2024, 29, 4816. https://doi.org/10.3390/molecules29204816

P.23_ Design, synthesis and biological evaluation of Pan-hCoV helicase inhibitors

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Helicase of human coronaviruses (hCoVs) uses energy from nucleotide triphosphate hydrolysis to catalyze the unwinding of double-stranded DNA or RNA in a 5′ to 3′ direction. Due to its highly conserved sequence and essential role in viral replication, it is a promising and attractive target for drug development aimed at treating various hCoVs, which can cause mild respiratory illnesses or severe and potentially fatal diseases. [1]
In this study, with the goal of identifying novel potential inhibitors of Pan-hCoV helicase, we explored available crystallographic structures of SARS-CoV-2 helicase [2, 3] to rationally design a library of compounds. These compounds were synthesized and characterized using structural (single-crystal X-ray diffraction) and spectroscopic (NMR, MS) techniques, and subsequently subjected to biological evaluation. All compounds demonstrated the inhibition of both SARS-CoV-2 helicase-

related enzyme activities, specifically NTPase and unwinding activities, with IC_{50} values in the low micromolar range. Several compounds also inhibited SARS-CoV-2 replication with low EC_{50} values and exhibited no significant cytotoxicity (CC50). Moreover, some of the most potent compounds showed substantial antiviral effects against HCoV229E and MERS-CoV, positioning them as promising Pan-hCoV helicase inhibitors. These findings suggest that hCoV helicase is a valid target for developing new treatments for SARS-CoV-2 and other hCoVs, potentially addressing future emerging or re-emerging coronavirus-related diseases.

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2. Newman, J.A., et al. Nature Communications, 2021. 12(1): p. 4848.
3. Chen, J., et al. Nat Struct Mol Biol, 2022. 29(3): p. 250-260.

P.24_ Unveiling the potential of pyrazoles as West Nile NS3 helicase inhibitors

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West Nile virus is a vector born- RNA virus belonging to the Flaviviridae Family, genus Flavivirus. It represents a global health concern because it can cause serious diseases such as encephalitis, meningitis, and severe muscle weakness. To date, neither vaccines nor antiviral treatments are available. [1]
The 11-kb positive-sense, single-stranded RNA (ssRNA) genome is translated into a single polyprotein, which is subsequently cleaved into three structural proteins (C, prM/M, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Among them, NS3 (72-kDa) is the second-largest viral protein after NS5 in the flavivirus genome and represents the motor protein in-volved in the double-stranded RNA (dsRNA) separation during viral replication. NS3 contains a serine-protease domain at its N terminus and an ATP-driven helicase and RNA triphosphatase at its C-terminal end. For its role in viral replication, NS3 represents a valid druggable target. [2,3]
Computer-Aided Drug Design (CADD) approaches allowed the identification of a series of compounds characterised by the 4,5 dihydropyrazole scaffold capable of inhibiting the $NS3^{hel}$ activity at low µM concentration. Starting from these results, an optimisation process, involving several structural modifications, with the aim to improve the activity and decrease the toxicity is in progress.

P.25_ A combined computational and biochemical approach for the identification of ZIKV $NS3^{pro}$ potential allosteric inhibitors

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Zika Virus (ZIKV) belongs to the Flavivirus genus, and the infections it causes, transmitted by Aedes species mosquitoes pose an ongoing threat to public health. The ZIKV genome contains NS2B-NS3 protease (NS2B-$NS3^{pro}$), which is essential for viral replication that cleaves the viral polyprotein to yield structural and non-structural proteins.[1] The $NS3^{pro}$ is a chymotrypsin-like characterised by the catalytic triad – S135, H51, D75 – at the N^{-ter} region. The structural homology between the active centre of $NS3^{pro}$ and various host serine proteases with important biological activities and the inefficacy of covalent peptide derivatives inhibitors made developing selective competitive inhibitors

challenging, making allosteric inhibitors the preferred option. Currently, neither medicines nor vaccines are commercially available.
The drug design approach involved the application of Molecular Dynamic simulations (MDs) of the ZIKV NS2B-NS3^{pro} in complex with NSC86314, and cluster trajectories – 1 μs x 3 x 2 systems – to take into account the protein-ligand flexibility. A double approach was applied, the former was to generate a dynamic pharmacophore, called Dynophore using the information obtained from MDs, and the latter was to model a Structure-Based (SB) pharmacophore.
The generated pharmacophore models were used as queries in a pharmacophore-based Virtual Screening (VS) campaign. The FDA-approved compounds and SPECS databases were screened to identify new hit scaffolds. Among 16 compounds selected for biological evaluation, according to their pharmacophore-fit value, 3 exhibited an IC_{50} value below 30 μM.

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