5mC and 5hmC MODIFICATION OF HUMAN microRNAs: IDENTIFICATION OF WRITER ENZYMES AND ASSESSMENT OF THE EFFECTS OF THEDE EPITRANSCRIPTOMIC MODIFICATIONS ON microRNA ACTIVITY.

VALERIO FULCI
RESEARCH AREA: Genetics, biology and pathophysiology of eukaryotes

Department of Molecular Medicine
valerio.fulci@uniroma1.it

Our research group focused its research on human microRNAs, investigating the biogenesis, mechanisms of action and physiologic relevance of these small RNA molecules in cancer.
In the current project we exploited two main models of cancer to unravel the role of microRNA epitranscriptomic modification. On the one hand we investigated the miR-34 → p53 axis in an established cell line model (HCT116) derived from colon cancer. This model represents a well characterised pathway whose relevance has been widely validated.
On the other hand we looked for m5C modified microRNAs in three cell lines (BxPC-3, Panc-1 and Suit-2) which model different stages of pancreatic adenocarcinoma progression. Pancreatic adenocarcinoma currently represents one of the most aggressive human tumours with a very poor prognosis, increasing the body of knowledge about this disease is a key achievement towards the development of novel therapies. We are also validating the enzyme(s) responsible for the deposition of m5C on human micrornas.
Our research project had two major objectives:

1) **Understanding the putative role of m5C in modulating microRNA stability/processing/activity**

To address the first point we focused on two miRNAs which, based on our bisulfite-seq data, carry one (miR-34a) or several (miR-16) m5C residues, some of which in the "seed" region of the mature miRNA. We took advantage of synthetic m5C modified mimic RNA oligonucleotides corresponding to miR-34a, carrying or not the m5C modification. We introduced these oligonucleotides into HCT116 cell line and measured both the stability of the synthetic miRNAs and the effects on direct and indirect known targets of miR-34a. Our data suggest that m5C modification of mature miRNAs does not affect the stability of the molecule and/or loading on AGO proteins. For miR-34a we observed overall comparable effects on direct targets in HCT116 cells. Similar experiments on miR-16 are ongoing.
In a complementary approach we chose to look for further m5C modified microRNAs in cancer. We have performed a bisulfite-seq screening of three different pancreatic adenocarcinoma cell lines. Our data highlight m5C deposition on several miRNAs. We decided to focus on miR-193b, which has been reported to act as a tumour suppressor miRNA by downmodulating K-ras expression in pancreatic cell lines. Our data show that

miR-193b carries a m5C modification adjacent to the "seed" region. We are currently working to verify whether m5C at this position affects the ability by miR-193b to repress K-ras expression and/or miR-193b stability/processing in pancreatic cancer cell lines.

2) **Identification of the enzyme(s) responsible for m5C deposition on human miRNAs**

To address this second issue we have generated stable Knock-Down (KD) cell lines for the candidate enzymes by stable introduction of siRNA-encoding ectopic DNA. microRNAs extracted from these cell lines were analysed by bisulfite-miRNA-seq.
Our analysis highlights that NSUN2 enzyme plays a role in the deposition of m5C on a subset of microRNAs. Indeed, the overall rate of m5C modification is reduced in NSUN2 KD cells. In particular NSUN2 loss specifically affects the m5C modification of a subset of miRNAs, including miR-126-3p (P-value 1 E-18), miR-30c-2-3p (P-value 1.8 E-5) and miR-744-5p (P-value 6E-4). On the other hand, DNMT2 KD has a milder effect on overall m5C deposition on microRNAs. However, it is interesting to highlight that DNMT2 KD significantly reduced miR-126-3p m5C deposition. These data suggest that the C residue in position 6 of miR-126-3p could be methylated by both NSUN2 and DNMT2. We plan to obtain a double KD cell line to assess whether depletion of both enzymes results in complete loss of m5C modification of this microRNA.

Significance and Future Work

Our results suggest that miR-34a m5C modification has a limited effect on target repression, with a slight increase of its activity which does not attain statistical significance. These results may be due to the modification of the "control" mimic RNA upon transfection in the HCT116 cell line. Furthermore, our data highlight that KD of single enzymes might not be sufficient to attain a full loss of m5C, most likely due to residual enzymatic activity which is expected in the KD approach.
To address these two issues (possible m5C deposition on "control" mimi RNA upon transfection in cells; putative limitations of the Knock-Down approach for identification of m5C writer enzymes) we will introduce minor modification in our project.
On the one hand, to verify whether "control" miRNA undergo methylation upon transfection in human cell line, we will check m5C modification status of transfected miRNAs 48 hrs after transfection by RNA Immunoprcipitation with m5C specific antibodies. We therefore aim to repeat experiments in RNA-methyltransferase KO cells (as described in the next paragraph). These modification will be implemented for experiments involving miR-34a, miR-16 and miR-193b.
On the other hand, to improve the significance of our results we will extend our project to obtain Knock Out (KO) (rather than KD) clones of the HCT116 cell line for the candidate enzymes. We have already designed and obtained the CRISPR constructs by cloning gRNAs in the pSpCas9(BB)-2A-Puro (PX459) V2.0 plasmid and, following transfection of HCT116, clone selection is currently ongoing.

In the second year of the project we are confident to be able to complete all the ongoing Tasks. In particular, we will screen by NGS the methyltransferase KO clones to further verify preliminary data obtained by KD. We expect to attain a higher sensitivity and therefore to be able to identify a larger number of microRNAs which are specifically methylated by each methyltransferase.
Furthermore, we will assess in the second year the role of m5C modification on miRNA processing, by providing cells with synthetic pre-miRNAs carrying the m5C modification (as compared to unmodified pre-microRNA) and assessing processing of the mature miRNA, using also ex vivo assays as detailed in our project proposal.

Research Group
Elisa Lorefice (-April 2022), PhD student
Francesco Greco, PhD student

Collaborations
Leandro Castellano, University of Sussex (UK)

CELLULAR AND MOLECULAR NETWORKS INVOLVED IN THE MAINTENANCE OF NEUROMUSCULAR JUNCTION

LUCA MADARO
RESEARCH AREA: Genetics, biology and pathophysiology of Eukaryotes

Department of Department of Anatomical, Histological, Forensic & Orthopaedic Sciences
luca.madaro@uniroma1.it

State of the Art

Skeletal muscle is a complex organ in which several mononucleated cell populations organize within specific anatomic compartments. These domains -nerves, connective, and vasculature - act altogether in coordination with muscle fibers to ensure the execution of contraction. In response to acute injury specific resident muscle populations, activate and contribute to regeneration by regulating muscle stem cell activity or cooperating with immune cells recruited from the circulatory system (Neutrophils, Macrophages) (Oprescu et al., 2020). However, muscle regeneration is not limited to myofiber repair, in order for the muscle to be functional all the different compartments need to be repaired. Indeed, voluntary contraction can only be restored through the establishment of new neuromuscular junctions (NMJs) on the newly formed myofibers (Liu and Chakkalakal, 2018). Innervation not only enables voluntary muscle control but also contributes to the maintenance of tissue homeostasis by regulating the balance between anabolic and catabolic responses(Sartori et al., 2021).

Dysfunction in nerve-muscle cross-talk is a pathological feature of many nerumuscular diseases such as Amyotrophic Lateral Sclerosis (ALS). Dissecting the molecular and cellular network that drives muscle denervation could help to understand the progression of ALS and identify new molecular targets to counteract the disease.

To this end, we combined several next-generation assays to dissect the molecular and cellular network that controls muscle innervation. Recently we define the transcriptional profile of the different anatomical compartments in skeletal muscle and set the basis for the understanding of differential fiber sensitivity to atrophy and nerve dependent gene expression using Visium Spatial Transcriptomic (ST) assay.

Spatial transcriptomic clustering reveals functional and structural organization of muscle tissue

Using ST we correlated the distribution of genes in unbiased predicted clusters with functionally characterized muscle areas. Tibialis Anterior (TA) muscle is characterized by a glycolytic cortex, in which muscle fibers are predominantly lightly stained with NADH-TR, and by a darker oxidative core (Figure 1A). Indeed, staining intensity directly correlates with the number of mitochondria within a muscle fiber and reveals the characteristic pattern fiber types. Interestingly, whereas genes Cluster 1 was associated

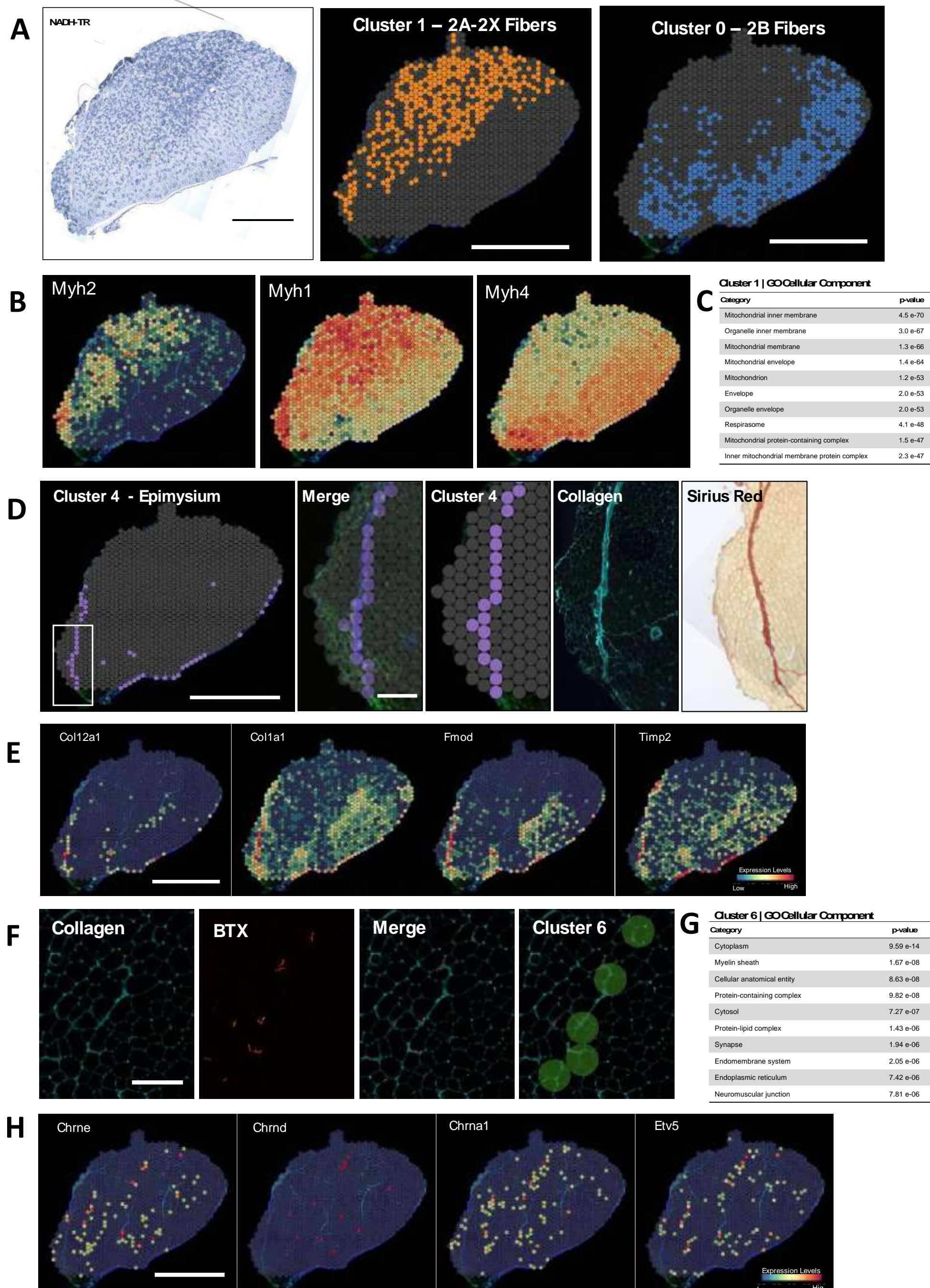
with the deeper oxidative core of the muscle, Cluster 0 overlapped with the glycolytic cortex of the tibialis containing mostly 2B fibers (Figure 1A). Indeed, Gene Ontology (GO) analyses of Cluster 1 genes revealed the enrichment of terms associated with mitochondrial structure and respiration and, as expected, cells in the two clusters expressed canonical markers of specific fiber types (Figure 1B-C). For example, myosin heavy chain 2 (*Myh2* – characteristic of 2A fibers), is spatially restricted to Cluster 1, while the fast twitch glycolytic myosin isoform 4 (*Myh4*) and the associated fast isoform cosegregated into Cluster 0 (Figure 1B). Interestingly enough in Cluster1 we detected also the expression of myosin heavy chain 1, (Myh1) a hallmark of 2X fibers (Figure 1B). We speculated that, given their phenotype, intermediate between 2A and 2B in terms of contraction velocities, and mitochondrial activity (Schiaffino and Reggiani, 2011) , these fibers could also be present in Cluster1.

Next, we analyzed the molecular signature of the other two clusters that presented a clear spatial distribution. Upon GO analysis, both Clusters 4 display an enrichment in ECM components. Indeed, Cluster 4 clearly identified the epimysium surrounding the muscle (Figure 1D). Almost perfect overlap was obtained between the localization of Cluster4 and the localization of collagen 1 surrounding the muscle (validated by Immunofluorescence and Sirius red staining - Figure 1D - inset). As expected, the genes enriched in this cluster are extracellular matrix components such as collagen subunits (Col12a1 and Col1a1) and matrix-associated molecules (Fmod and Timp2) (Figure 1E). Cluster 6 spots, on the other hand, nicely colocalized with acetylcholine receptor across muscle sections (stained with Alexa 594-conjugated BTX). In line with the spatial pattern GO analysis of genes enriched in of Cluster6 contained terms associated with NMJs and synapses (Figure 1G). Lastly, representative genes of this cluster encode acetylcholine receptor subunits (Chrne, Chrnd, Chrna1) or transcription factors known to be expressed in subsynaptic nuclei (Etv5)(Kim et al., 2020) (Figure 1H).

Figure 1

A) *NADH-TR staining of CTR muscle (TA) (scale bar, 1 mm); cluster 0 and cluster 1 patterns display an overlap with NADH-TR low- and NADH-TR high- expressing fibers (scale bar, 2 mm).*
B) *Relative expression levels of fiber-type- specific marker genes from the ST data projected over the tissue space (scale bar 2mm).*
C) *Cellular Component and Biological Process Gene Ontology analyses of Cluster 1*
D) *Spatial distribution of cluster 4 (left) (scale bar, 2 mm). Overlay of cluster 4 distribution over tissue space, collagen-1 staining of the region highlighted, and Sirius red staining of serial section (scale bar, 100 mm).*
E) *Relative expression levels of genes enriched in cluster 4 (scale bar, 2 mm).*
F) *Immunostaining for collagen-1 and bungarotoxin respectively (and merge) and overlay with cluster 6 (scale bar, 100 mm).*
G) *Cellular Component and Biological Process Gene Ontology analyses of Cluster 6*

Relative expression levels of NMJ-specific marker genes from the ST data projected over the tissue space (scale bar, 2 mm).



Cluster 1 | GO Cellular Component

Category	p-value
Mitochondrial inner membrane	4.5 e-70
Organelle inner membrane	3.0 e-67
Mitochondrial membrane	1.3 e-66
Mitochondrial envelope	1.4 e-64
Mitochondrion	1.2 e-53
Envelope	2.0 e-53
Organelle envelope	2.0 e-53
Respirasome	4.1 e-48
Mitochondrial protein-containing complex	1.5 e-47
Inner mitochondrial membrane protein complex	2.3 e-47

Cluster 6 | GO Cellular Component

Category	p-value
Cytoplasm	9.59 e-14
Myelin sheath	1.67 e-08
Cellular anatomical entity	8.63 e-08
Protein-containing complex	9.82 e-08
Cytosol	7.27 e-07
Protein-lipid complex	1.43 e-06
Synapse	1.94 e-06
Endomembrane system	2.05 e-06
Endoplasmic reticulum	7.42 e-06
Neuromuscular junction	7.81 e-06

Spatial transcriptome clustering analysis of transiently denervated muscle

Once correctly annotated the different clusters in the unperturbed muscle we proceeded to investigate their response to reversible denervation. Sciatic nerve compression is characterized by a stereotypical induction of the atrophic response that is resolved upon reinnervation (Magill et al., 2007). Complete denervation of TA and EDL is observed three days after injury (not shown) with the consequent activation of the muscle-specific ubiquitin ligases Atrogin-1 (Fbxo32) and MuRF1 (Trim63) in both muscles. Interestingly, we noticed that the induction Atrogin-1 and Murf1 was primarily localized in the glycolytic cortex of the TA muscle (Figure 2A). This observation suggests that the distribution pattern of the atrophic response depends on the muscle fiber composition, consistent with data in the literature indicating that glycolytic muscle fibers are more susceptible to muscle atrophy than oxidative muscle fibers (de Theije et al., 2015; Wang and Pessin, 2013).

Nerve-dependent polyamine synthesis-related enzyme pattern across muscle

Obtaining a detailed map of the spatial localization of specific genes in muscle could be pivotal for pinpointing new signaling pathways involved in the pathophysiology of the tissue. To this end, the possibility of identifying the specific distribution of certain pathways in functionally different areas of muscle will be of particular interest. As a proof of concept, we identified the spatial distribution of the genes encoding Smox and Amd1, enzymes related to polyamine (PA) synthesis (Figure 2B), which showed a pattern reminiscent of the distribution of glycolytic fibers in the TA. Specifically, Amd1, Amd2 and Smox expression profile was largely overlapping the glycolytic cortex of the muscle (Figure 2B-C) with a clear enrichment in the 2B Fiber cluster when compared to 2A-2X cluster.

It is noteworthy that, concomitantly with a with a reduction in overall expression in the glycolytic cluster, we observed a partial loss of the spatial restriction of these genes (Figure 2B). Intriguingly, at 30 days after the induction of nerve damage- when innervation is restored- Amd1, Amd2 and Smox expression in the muscle is still decreased compared to CTR levels. This could suggest that more time or more extensive maturation of NMJs is needed to re-establish the correct expression of these markers.

We validated this observation through laser microdissection; indeed, while the expression of Amd1, Amd2 and Smox was high in the glycolytic portion of the TA in control tissue, after denervation, due to their reduced expression in glycolytic fibers, the relative levels of these genes were similar between the glycolytic and oxidative fractions of the muscle (Figure 2C). We speculated that such change in expression levels could result in an imbalance in the PA pathway. The decarboxylation of S-adenosylmethionine (SAM), catalyzed by Amd1/Amd2, produces an aminopropyl group that acts as a substrate together with Putrescine for the Spermidine synthase and subsequently to form Spermine. Thus, reduction of Amd1/Amd2 expression may results in Putrescine accumulation. Indeed, as shown by gas chromatography-mass spectrometry (GC-MS) quantitative analysis, we observed a dramatic change in polyamine ratio 3 days after

denervation (Figure 2D). A mean 25-fold increase of Putrescine was revealed as results of Amd1 limitation.
Collectively, these data show how ST could be used to identify variation in the spatial patterning of specific genes and pathways in skeletal muscle – as in example occurs to the PA pathway as result of denervation. Further studies will define whether a similar alteration is a feature of ALS progression and whether restoring BP balance can delay disease progression and denervation.

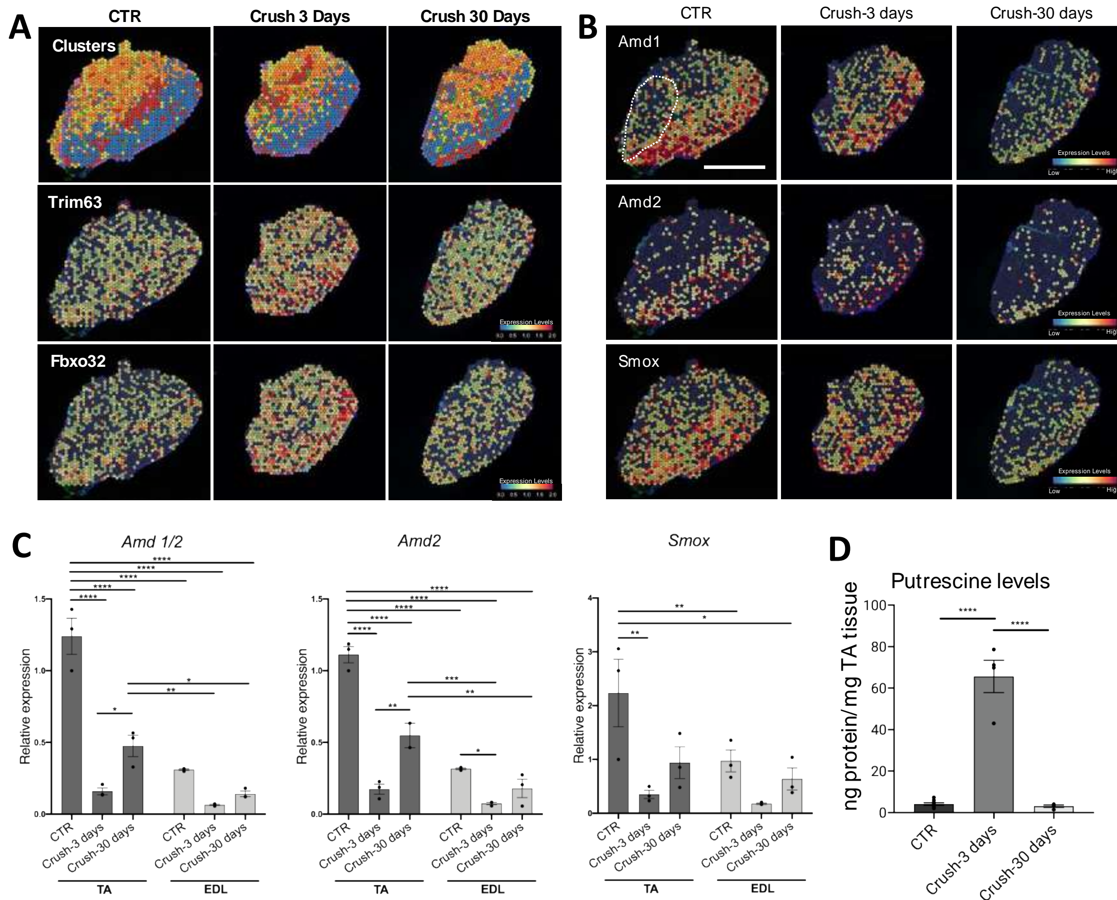


Figure 2

A. *Cluster distribution and relative expression levels of Fbox32 and Trim63 transcripts during denervation-reinnervation process (scale bar, 2 mm).*

B. *Cluster 0 distribution and relative expression levels of spermidine pathway component (Amd1, Amd2, and Smox); scale bar, 2 mm (dotted line highlights EDL muscle).*

C. *Relative expression of Amd1/2, Amd2, and Smox during denervation in TA and EDL muscle (n = 3, values represent mean ± SEM, *p < 0.05, **p < 0.01,***p < 0.001, ****p < 0.0001, by two-way ANOVA Tukey's multiple-comparison test).*

D. *GC-MS quantitative analysis of putrescine levels in control (CTR) and denervated (crush-3 and crush-30 days) muscles (n = 8 CTR, n = 4 denervated [crush-3 and crush-30 days]; values represent mean ± SEM by ****p < 0.0001, by unpaired two-tailed t test).*

Publications

Chiara D'Ercole, Paolo D'Angelo, Veronica Ruggieri, Daisy Proietti, Laura Virtanen, Cristina Parisi, Carles Sanchez Riera, Alessandra Renzini, Alberto Macone, Marta Marzullo, Laura Ciapponi, Davide Bonvissuto, Claudio Sette, Lorenzo Giordani and Luca Madaro ***Spatially resolved transcriptomics reveals innervation-responsive functional clusters in skeletal muscle***. Cell Reports. 2022, 20;41(12):111861. IF: 9.995

Research Group

Madaro Luca (Researchers)
Carles Sanchez (Postdoc)
Chiara D'Ercole (PhD student)
Veronica Ruggieri (PhD student)

Collaborations

Giordani Lorenzo , Sorbonne University (Paris, France)
Macone Alberto (Sapienza Università di Roma)
Laura Ciapponi (Sapienza Università di Roma)
Claudio Sette (Università Cattolica di Roma)

ROLE OF METABOLIC NICHE IN THE METASTATIC POTENTIAL OF LUNG CANCER CELLS

ALESSIO PAONE
RESEARCH AREA: Novel therapeutic interventions

Department of Biochemical Sciences
A. Rossi Fanelli
alessio.paone@uniroma1.it

During the first year of our research, we have primarily focused on addressing the AIM1 of the project. As outlined in the proposal, we have generated substantial data to help elucidate the mechanisms that govern the development of brain metastases originating from lung cancer cells. We are also actively working on the remaining objectives, but the data for these tasks are not yet sufficiently clear or conclusive to be included in this report. Below, you can find the title of each task as originally presented in the project, along with the results obtained pertaining to each specific task.

(AIM1) Determine the molecular mechanism by which Ala/Gln/Glu and Ser/Gly in the extracellular environment control migration of lung cancer cells in vitro.
TASK 1.1 Dissecting the pathway(s) involved.
Considering the expertise of our laboratory, we have begun dissecting the pathways that regulate the increase in chemotaxis in cancer cells, mediated by extracellular amino acids, by studying Serine/Glycine metabolism, a leading subject in our lab. Using RNA interference capable of specifically targeting Serine Hydroxymethyl Transferases (SHMT1 or SHMT2), the proteins responsible for the interconversion of serine to glycine and vice versa, we demonstrated that SHMT1, and then the cytoplasmic serine, controls the migratory ability of lung cancer cells in the presence of increasing doses of both amino acids in the microenvironment. Figure 1 provides a summary schematic of this set of experiments.

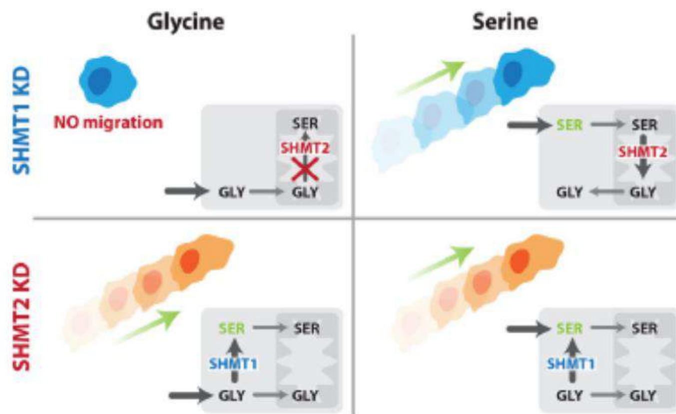


Figure 1. Scheme summarizing the results obtained by knocking down SHMT1 (SHMT1 KD) or SHMT2 (SHMT2 KD) on cytosolic Ser levels and cell motility.

TASK 1.2 Dissecting the role of energy metabolism and redox stress.

We conducted rescue experiments with extracellular ATP, antioxidants, and hypoxanthine, demonstrating that both ATP increase and cytoskeleton remodeling are crucial for cell motility. Our findings indicate that reduced cytoplasmic serine levels lead to decreased ATP and increased ROS production, impairing cell motility. To investigate the impact of decreased intracellular serine levels on ATP in lung cancer cell lines, we used minimal medium (MEM) with or without serine or glycine + formate supplementation. We observed a significant decrease in ATP levels and an increase in ROS formation when lung cancer cells were grown in MEM without amino acids, compared to complete medium (RPMI) (Fig. 2A, B). Serine or glycine supplementation reversed this effect, increasing ATP (Fig. 2A) and decreasing ROS production (Fig. 2B). Interestingly, supplementation with mouse brain extracellular fluid (BEF) also had similar effects (Fig. 2A, B). Using inhibitors of glycine or serine uptake, we found that the metabolic rescue was significantly, but not fully, impaired (Fig. 2A–D). To further explore the link between cytoplasmic serine levels and cellular ATP, we studied mitochondrial respiration by evaluating oxygen consumption rate (OCR) and glycolysis by measuring extracellular acidification rate (ECAR) in A549 cells grown in RPMI or serine-enriched RPMI. Adding serine significantly increased both respiration and glycolysis (Fig. 2E, F), while treatment with serine and 4LFPG inhibitor reduced the observed effect on ECAR, suggesting that cytoplasmic serine mainly modulates ATP production through glycolysis (Fig. 2F). The influence on mitochondrial respiration was revealed upon stressor treatment, with a significantly reduced maximal respiratory capacity in 4LFPG-treated cells compared to control (Fig. 2E). To further validate our hypothesis, we performed rescue experiments with extracellular ATP, antioxidant molecules (GSH and NADPH), and hypoxanthine. As shown in Fig. 2G, supplementation with ATP, GSH, or NADPH partially rescued migration, while hypoxanthine administration fully recovered cell motility, indicating that both ATP increase and ROS reduction are required for cell motility. These data demonstrate that decreased

cytoplasmic serine levels result in reduced ATP production and increased ROS production, impairing cell motility.

TASK 1.3 Is AMPK the effector of the cellular response triggered by aa uptake inhibition?
Our findings suggest that pulmonary adenocarcinoma cells activate a defensive mechanism in response to modest reductions in cytoplasmic serine levels, shutting down non-essential functions like migration while preserving essential functions like proliferation (Fig. 3D, E). AMP-activated protein kinase (AMPK) serves as a key modulator that senses increased ROS and decreased ATP levels, affecting cellular activities. To examine the involvement of AMPK in inhibiting cell migration due to serine/glycine uptake inhibitors SARC or 4LFPG, we assessed AMPK activation by measuring the phosphorylation level of AMPK Thr172 per total protein levels ratio (pThr172AMPK/AMPK) through western blot analysis. Both drugs increased the phosphorylated form of the protein from 5 to 15 minutes, indicating enzyme activation (Fig. 3A, B). Using dorsomorphin, a specific AMPK inhibitor, we demonstrated AMPK's direct involvement in SARC/4LFPG-mediated inhibition of cell motility. At 2 µM concentration, dorsomorphin completely reversed the inhibitory effect induced by SARC

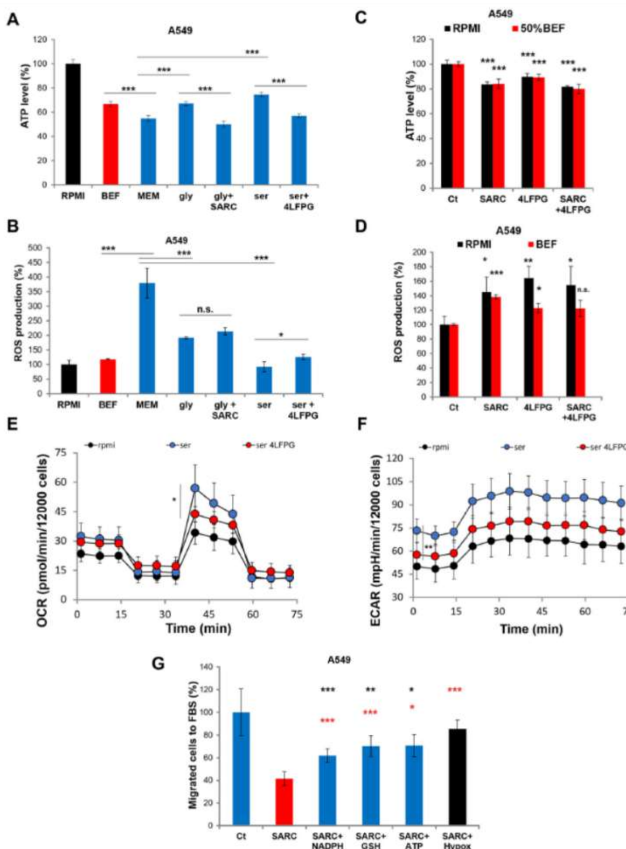


Figure 2. Cytosolic serine levels controlled by SHMT1 modulate ROS formation and ATP/energy profile. Effect of nutrients on ATP and ROS levels in A549 cells. The effect of 400 µM Ser ± 4LFPG or Gly ± SARC supplementations, on ATP levels (A) and ROS production (B) in A549 cells grown in MEM as compared to cells grown in complete medium RPMI or 50% brain extracellular fluid (BEF). Effect of the inhibition of Ser/Gly uptake on ATP (C) and ROS levels (D) in A549 cells grown in RPMI complete (10% FBS) or 50% BEF in MEM. The levels of the indicated parameters were assayed after incubation of the cells with 100 µM of 4LFPG and/or SARC. ATP concentration measured for RPMI (A) and Ct (C) are 29.4 ± 3.4 nmol/mg protein and 19.6 ± 1.9 nmol/mg protein, respectively. For all the experiments in (A–D) n = 4. OCR (E) and ECAR (F) analysis (n = 4) of A549 cells in RPMI (black) or RPMI with increased serine concentration (385 µM, blue in the figure) and in combination with 4LFPG (red); a representative experiment is shown (data are the mean of at least five wells ± SD). Asterisks refer to P values for serine vs. 4LFPG. P values for RPMI vs. serine samples are < 0.05; **P < 0.01; ***P < 0.005

(Fig. 3C), while migration remained unaffected by AMPK inhibition in the absence of SARC. AMPK's role in inhibiting cell migration is not well understood, but it is known to attenuate the production of filopodia and lamellipodia, subcellular structures involved in microenvironment exploration and movement. We used phalloidin, which specifically binds to filamentous actin, to further investigate the connection between cytoplasmic serine starvation and filopodia/lamellipodia formation. SARC-treated A549 cells showed severely affected lamellipodia and filopodia structures (Fig. 3D), with a reduced cell surface (Fig. 3E). Co-treatment with SARC and ATP, NADPH, GSH, hypoxanthine, or dorsomorphin increased lamellipodia and filopodia production (Fig. 6D) and cell surface (Fig. 3E), supporting our hypothesis that podia formation is inhibited by ROS upregulation and ATP reduction in an AMPK-mediated process.

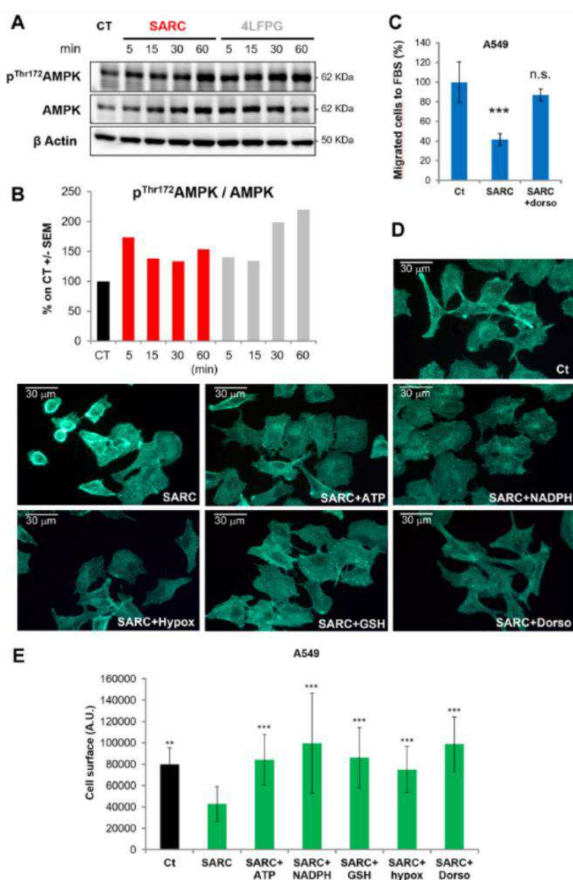


Figure 3. AMP kinase is the sensor of serine starvation modulating cell migration. A Western blot images showing a time course of p-AMPKThr172 and total AMPK protein levels in presence of 100 µM SARC or 4LFPG; actin was used as loading control. B Densitometric evaluation of the p-AMPKThr172/ AMPK ratio. Data are expressed as percentage of CT set as 100%, the experiment has been repeated three times with similar results. C Migratory ability of A549 cells to serum in the presence of sarcosine and dorsomorphin. Cells pretreated for 3 h with 25 µM SARC ± 2 µM dorsomorphin (dorso) and nonpretreated control cells were tested in Boyden chamber assay (Ct) (n = 3). D F-actin visualization using phalloidin in A549 cells, after incubation for 24 h with each of the indicated compounds (25 µM SARC plus 25 µM ATP or hypoxanthine (hypox), 200 µM GSH, 150 µM NADPH, or 0.5 µM dorso). E Cell surface analysis of three images taken at the end of the experiment shown in (D); average ± SD is shown. $^{**}P < 0.01$; $^{***}P < 0.005$.

Publications

Chiara Scribani Rossi, Laura Barrientos-Moreno, Alessio Paone, Francesca Cutruzzolà, Alessandro Paiardini, Manuel Espinosa-Urgel, Serena Rinaldo. ***Nutrient Sensing and Biofilm Modulation: The Example of L-arginine in Pseudomonas***. *international journal of molecular sciences*. 2022 N 23 e Vol 8. e IF: 6,208

Research Group

Sara Di Russo PhD student**, Amani Bouzidi Postdoc, Agnese Riva** PhD student;

Collaborations

Prof. Serena Rinaldo, Dept of Biochemical Sciences A. Rossi Fanelli, Sapienza University of Rome

EXPLORING NEW FUNCTIONS OF THE NIJMEGEN BREAKAGE SYNDROME GENE IN CEREBELLAR DEVELOPMENT AND CARCINOGENESIS

MARIALAURA PETRONI

RESEARCH AREA: Genetics, biology and pathophysiology of eukaryotes

Department of Molecular Medicine
marialaura.petroni@uniroma1.it

At the time of the initial submission, preliminary results indicated that full NBS1-KO impairs MB development both in SmoA1 mice and in a SHH-driven tumor allograft, revealing an unpredicted function of NBS1 in SHH-pathway and tumorigenesis. These results have now been completed and published (Petroni et al., published paper 1, see below).
On the base of these results and of preliminary experiments indicating a possible involvement of NBS1 at the primary cilium (PC) we aimed to evaluate:

1) whether the outcome of NBS1-KO on the PC is directly responsible for SHH-pathway inhibition;
2) whether inhibition of the SHH-pathway due to NBS1-KO is responsible for the observed defects in cerebellar development and SHH-MB inhibition;
3) whether NBS1 function on PC regulation is dependent on DDR activation;
4) the molecular mechanism underpinning the effect of NBS1-KO on ciliogenesis;
5) whether human NBS1 mutations result in PC defects.

To this end, we generated and extensively analyzed the Ptch1/Nbn$^{GCP\Delta}$ mouse, with interesting results which are summarized as follows.

Nbn^{KO} blocks SHH-dependent MB development (TASK1: Generation and analysis of GCP-specific NBS1-KO mouse model to dissect NBS1 role on ciliogenesis, SHH-pathway and cerebellar development/transformation; Task 1.1 and 1.3)

Given that Granule Cell progenitors (GCP)-specific inactivation of the Ptch1 (Ptch1$^{GCP\Delta}$) inhibitory receptor of the SHH pathway leads to MB development with 100% penetrance, we comparatively analyzed Ptch1$^{GCP\Delta}$ (n = 28), Ptch1/Nbn$^{GCP\Delta}$ (n = 28) and Nbn$^{GCP\Delta}$ mice for head doming, ataxia, or weight loss as potential markers of MB development. While Ptch1$^{GCP\Delta}$ mice developed symptomatic MB between 44 and 120 days, with an average latency of 84 days, the majority of Ptch1/Nbn$^{GCP\Delta}$ mice (64%) displayed the characteristic indicators of a progressed MB starting from day 92, some animals reaching up to 217 days, with an average latency of 101 days (Fig. 1A, B). We never observed MB signs in Nbn$^{GCP\Delta}$. As expected, P7 Ptch1$^{GCP\Delta}$ mice exhibited bigger cerebella and thickening of the EGL when compared to the WT littermates (Fig 1C). Consistently, at P21, when all the GCPs in WT mice had migrated and differentiated to complete the IGL, Ptch1$^{GCP\Delta}$ cerebella maintained a severe hyperplasia with a dramatically thickened EGL (Fig. 1C),

which by 2 months of age had become full-blown MB encompassing and disrupting the entire cerebellar structure, with substantially undistinguishable lobes and folia (Fig. 1C). Conversely, the size of Ptch1/Nbn$^{GCP\Delta}$ cerebella was consistently reduced compared to Ptch1$^{GCP\Delta}$ at any age. Moreover, they showed a strongly reduced thickness of the EGL in the rostral lobes of the cerebella compared to Ptch1$^{GCP\Delta}$ mice at P7, mirrored by a hypoplasia of the rostral IGL at P21 and in the adult (Fig. 1C). At 2 months, the cerebella Ptch1/Nbn$^{GCP\Delta}$ mice appeared dramatically altered compared to WT mice, displaying crushed and deformed lobes (Fig. 1C). Most interestingly 100% of them developed MB in the caudal portion of the cerebellum (Fig. 1C, D) while their rostral portion displayed a more conserved appearance with a clearly distinguishable IGL. WB analysis of NBS1 protein expression from 2-month-old cerebellar explants surgically severed at the interface between the anterior and the posterior portions demonstrated high levels of NBS1 protein in both the rostral and caudal lobes of Ptch1$^{GCP\Delta}$ mice compared to age-matched WT adult cerebella. In contrast, we detected an efficient NBS1 depletion in the non-tumorigenic rostral portion of the Ptch1/Nbn$^{GCP\Delta}$ cerebellum, while the posterior tumor tissue showed high NBS1 expression (Fig. 1E), possibly resulting from a less efficient Math1-Cre-induced recombination of Nbn alleles in this part of the cerebellum. Additional experiments running in the lab should help us explain the differences between the anterior and posterior phenotype of the mice. MB growing in both genotypes were arranged in densely packed sheets of cells with hyperchromatic carrot-shaped nuclei and scant cytoplasm, which represent typical histopathological features of the SHH-MB subtype (Fig. 1F). Quantitative analysis of transcript expression of the main SHH targets, including Gli1, Gli2 and MYCN, indicated they were highly upregulated in both Ptch1$^{GCP\Delta}$ and Ptch1/Nbn$^{GCP\Delta}$ MBs (Fig. 1G), suggesting MBs developing in the two genetic backgrounds did not differ significantly in their molecular nature. Overall, these data suggested that GCP-specific NBS1 depletion (occurring in the anterior lobes) completely blocks SHH-MB development, implying that the NBS1^{KO} phenotype is dominant on that of Ptch1 loss.

GCP-specific Nbn^{KO} impairs cerebellar development and SHH pathway *in vivo/ex vivo* (extends and complement TASK1; part of the experiments were initially non planned, but were necessary due the above findings**)**

Nbn$^{GCP\Delta}$ mice were apparently indistinguishable from their WT counterparts, since they were viable, fertile, had no visible growth retardation and exhibited no readily detectable signs of cerebellar ataxia. Indeed, at variance with the Nbn-CNS-del model (Frappart PO et al, Nat Med 2005;11:538-44) these mice did not display clear microcephaly and maintained a roughly normal pattern of foliation of the cerebellum. Nonetheless, they clearly showed a significant reduction in the cerebellar area at both P7 and P21 developmental stages (Fig. 2A). In particular, EGL thickness and the number of GCPs appeared extremely diminished compared to the WT architecture at P7 and this was reflected by a reduced thickness of the IGL and granule cell (GC) number compared to WT on the mature Nbn$^{GCP\Delta}$ cerebellum at P21 (Fig. 2A, B). In situ immunofluorescence

assays on P7 cerebellar tissue showed that the cellular population lining the outer EGL in the anterior and posterior part of the WT cerebellar cortex were positive for Ki67 staining, consistent with the identity of proliferating GCPs. A similar condition was observed in the posterior region on the $Nbn^{GCP\Delta}$ cerebellum, while scant Ki67 signal was detected in the few GCPs of the anterior region. In addition, while only few cells stained positive for γH2AX in the WT cerebella, γH2AX signal was readily detectable in the $Nbn^{GCP\Delta}$ cerebella (Fig. 2C).

Overall, these results suggest that the GCP-specific Nbn deletion impacts on the developing cerebellar cortical architecture, by arresting GCP proliferation and/or by inducing apoptosis. Since GCP expansion wave occurring in early (P1-14) postnatal development of the mouse cerebellum is strongly dependent on the SHH pathway, we assessed whether its activity was altered. WB analysis of whole cerebellar extracts at P7, when SHH activity peaks, indicates downregulation of NBS1 protein and of the SHH downstream effectors *Gli1* and *MycN* in $Nbn^{GCP\Delta}$ cerebellar tissue when compared to WT counterparts (Fig. 3A). Since these observations could also be due to the lower number of GCPs in the $Nbn^{GCP\Delta}$ compared to the WT cerebella, we established primary cultures from freshly explanted GCPs at P7, stimulating them with the SHH agonist SAG for 48h. Importantly, we observed a relevant decrease in Gli1 and MycN protein levels and in the transcript levels of numerous SHH targets and proliferation markers by Microfluidic Card TaqMan Gene Expression Assay, in the $Nbn^{GCP\Delta}$ compared to the WT cultures (Fig. 3B,C). This was consistent with a significantly lower number of $Nbn^{GCP\Delta}$ cells being able to incorporate EdU after SAG treatment compared to the WT counterpart (Fig. 3D). Importantly, the inhibitory effect of Nbn depletion on the SHH pathway was further supported by the inhibition of the GLi1-Luc reporter in NIH3T3 Shh-Light II cells (Fig. 3E).

Overall, these data strongly indicate that the depletion of NBS1 abrogates SHH-MB by severely compromising the activity of SHH pathway and SHH-dependent proliferation.

Nbn KO leads to PC morphology alterations in GCPs *in vivo/ex vivo* (extends and complement TASK1)

Freshly explanted GCPs were dissociated and isolated from NbnΔ and WT cerebella at P7. Of interest, while the percentage of ciliated cells revealed no significant variation between the two genotypes, the length of the PC resulted considerably higher in NbnΔ GCPs compared to WT, as illustrated by the relative frequency curve of ciliary length distribution (Fig. 4A, B, C). To search for a similar condition also in vivo, we looked at PC morphology in formaldehyde-fixed P7 cerebellar sections of the $Nbn^{GCP\Delta}$ mouse by staining for Arl13b. The $Nbn^{GCP\Delta}$ cerebellum displayed a strongly disorganized Purkinje cell layer (as indicated by staining with the Calbindin), with a deeply irregular cellular lining and an extremely distorted dendritic branching (Fig. 4D). The length of the PC appeared considerably increased in the EGL of $Nbn^{GCP\Delta}$ cerebella when compared to the WTs (Fig. 4D). Under these conditions, the analysis of SHH targets confirmed a strong impairment of the pathway (see Fig. 3A,B,C). Collectively, our data indicate that Nbn^{KO}

severely alters the structural integrity of the PC and down-regulates SHH pathway, providing an explanation for the abrogation of Ptch1^{KO}-dependent MB in the double KO model, as postulated in the application.

NBS1 localizes at the basal body (TASK 2: Molecular mechanisms leading to PC dysfunction by NBS1-KO, TASK 2.1**)**
Several observations (reported in the grant application but also in the previous findings) suggested that NBS1 might be involved in maintaining the structure/function of the primary cilium. Importantly, we developed an IF protocol to co-stain for NBS1 and acetil-□-tubulin, which clearly demonstrated NBS1 localization at the basal body (BB) of the PC (Fig. 5A). This was also confirmed by isolation of centrosomal fractions in asynchronous (data not shown) or G0-arrested cells (Fig. 5D, see γtubulin enriched fractions) supporting the idea that NBS1 could have a functional role in the regulation of the BB/PC. Interestingly, similar localization data are available for both MRE11 and RAD50 (Fig 5B-C-D). Importantly, NBS1 continues to localize at the BB after both DNA Damage Response (DDR) activation (mediated by clastogenic drugs) and inactivation (mediated by ATM and CHK1 inhibitors) (data not shown).
Collectively, these data indicate that NBS1 localizes at the BB independently from the DDR status.

MRE11/RAD50/NBS1 (M/R/N) complex impairment, but not a general activation of the DDR, induces PC dysmorphisms (TASK 2: Molecular mechanisms leading to PC dysfunction by NBS1-KO, TASK 2.1**)**
At the time of the initial submission, preliminary results in MEF suggested that the phenotype observed upon NBS1 impairment was not a direct consequence of the DDR. Indeed, only mirin, but not other DDR inducers deregulated PC length. Now, we confirmed these data in RPE1 (Fig. 6A-B-C) and GCPs (data not shown). Moreover, we observed that neither the DDR inactivation, obtained by the treatment with ATM and CHK1 inhibitors, is able to lengthen the PC (Fig 6D-E-F), further indicating that NBS1-KO mediated PC deregulation is independent from the DDR status. Importantly, we also observed that MRE11 repression induced a PC phenotype almost identical to NBS1^{KO} (data not shown), supporting a never described role of the M/R/N complex in the control of PC structure/function.

P53 has a role in the NBS1-KO induced PC phenotype (TASK 2: Molecular mechanisms leading to PC dysfunction by NBS1-KO, TASK 2.3**)**
Several observations (reported in the grant application) suggested that P53 might be involved in the NBS1-KO induced PC phenotype. Importantly, p53^{KO} impaired PC phenotype and repression of the SHH pathway induced by NBS1^{KO} in GCPs (Fig. 7) and RPE1 (data not shown). However, whether the p53 function required to exploit the PC phenotype induced by MRN depletion is linked or independent from its DDR functions has not been dissected by these experiments and clearly deserves additional attention.

Significant discrepancy to planned TASK1-2-3

While we were able to accomplish in large part the proposed experiments, some important activities of TASK1 (1.2 and part of 1.3) could not be performed as planned. Indeed, the second mouse model described in our proposal (Gli2ΔN/NbnGCPΔ) could not be generated since the CLEG2 (Gli2ΔN) mouse, which we should have obtained from Dr. Andrzej A Dlugosz, is no longer available, having the owner lost the colony. We kept on pushing on Dr. Dlugosz to search for a colony available in his collaborator’s labs, with no success. Experiments with SuFu depletion and Gli2ΔN overexpression are ongoing to establish whether an alternative approach may lead to a similar information at least in vitro, before eventually planning an additional strategy for in vivo experiments. Moreover, some of the experiments described in TASK2 (TASK 2.2. and part of TASK 2.3) and TASK3 were postponed to give priority to the studies described above.

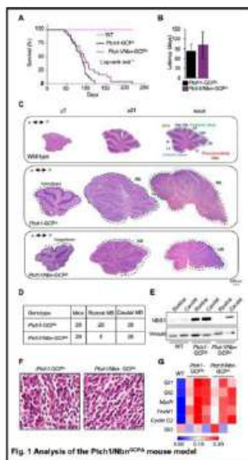


Fig. 1 Analysis of the Ptch1/Nbn$^{GCP\Delta}$ mouse model

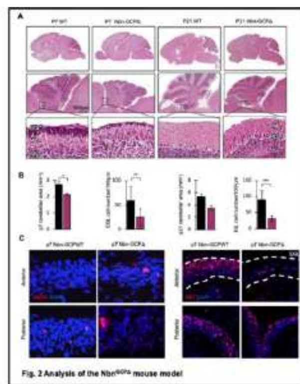


Fig. 2 Analysis of the Nbn$^{GCP\Delta}$ mouse model

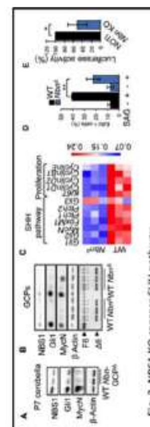


Fig. 3. NBS1 KO repress SHH pathway

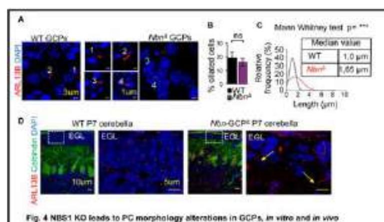


Fig. 4 NBS1 KO leads to PC morphology alterations in GCPs, *in vitro and in vivo*

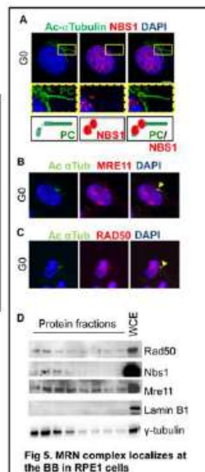
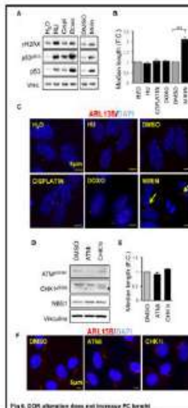


Fig 5. MRN complex localizes at the BB in RPE1 cells



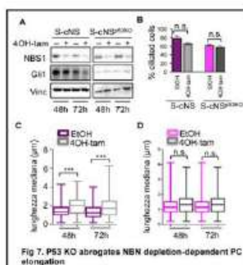


Fig 7. P53 KO abrogates NBN depletion-dependent PC elongation

Publications

Petroni M, Fabretti F, Di Giulio S, Nicolis di Robilant V, La Monica V, Moretti M, Belardinilli F, Bufalieri F, Coppa A, Paci P, Corsi A, De Smaele E, Coni S, Canettieri G, Di Marcotullio L, Wang ZQ, Giannini G. A gene dosage-dependent effect unveils NBS1 as both a haploinsufficient tumour suppressor and an essential gene for SHH-medulloblastoma. ***Neuropathology and Appl Neurobiology***. 2022 Oct;48(6):e12837. doi: 10.1111/nan.12837. IF:6.25

Research Group

Dr. Francesca Fabretti, Research fellow;
Dr. Veronica La Monica, PhD Student;
Dr. Francesca Polonara post-degree fellow

Collaborations

Dr. Simone De Panfilis, IIT CLN2S@Sapienza;
Prof. Alessandro Corsi, Department of Molecular Medicine, University of Rome La Sapienza.

MOLECULAR MECHANISMS DRIVING THROMBOINFLAMMATION IN COMMUNITY-ACQUIRED PNEUMONIA

LUCIA STEFANINI
RESEARCH AREA: Immuno-thrombosis

Department of Translational and Precision Medicine
lucia.stefanini@uniroma1.it

Respiratory tract infections are associated with an increased risk of acute cardiovascular events, in particular 10% of non-COVID-19 pneumonia patients and 20% of COVID-19 patients experience thrombotic complications. The risk of cardiovascular complications during infections is highest among the elderly who typically display a hyperactive innate response and an ineffective adaptive response. Thus, in this project we aim to characterize the platelet phenotype and the platelet-immune crosstalk in pneumonia patients in the hypothesis that platelets have both a pro-inflammatory and a pro-thrombotic role in respiratory tract infections.

Because of the pandemic, in the first period of the project we recruited mainly COVID-19 pneumonia patients. We enrolled 95 SARS-COV2-positive patients admitted at the Umberto I Hospital (Rome, Italy), 21 of which had received a complete course of vaccination more than 2 weeks prior infection (vaccinated COVID-19). Among unvaccinated COVID-19 patients, 36 required mechanical ventilation and were admitted to the intensive care unit (ICU COVID-19) and 38 were not critical and more heterogenous in terms of lung function (non-ICU COVID-19). Among the vaccinated COVID-19 patients only 3 were admitted to the ICU (males with type II diabetes, age>70 years). For comparison we recruited 48 SARS-CoV-2-negative internal medicine vaccinated patients matched for age, sex, and comorbidities. All participants gave written informed consent. Platelet phenotype, activation and leukocyte binding were measured directly in citrated whole blood by flow cytometry, within 30 minutes from withdrawal, as described previously (Flego et al., JTH 2022). The study was approved by the Ethics Committee of our institution (EC identifier: 5870; ClinicalTrials.gov Identifier: NCT04497402).

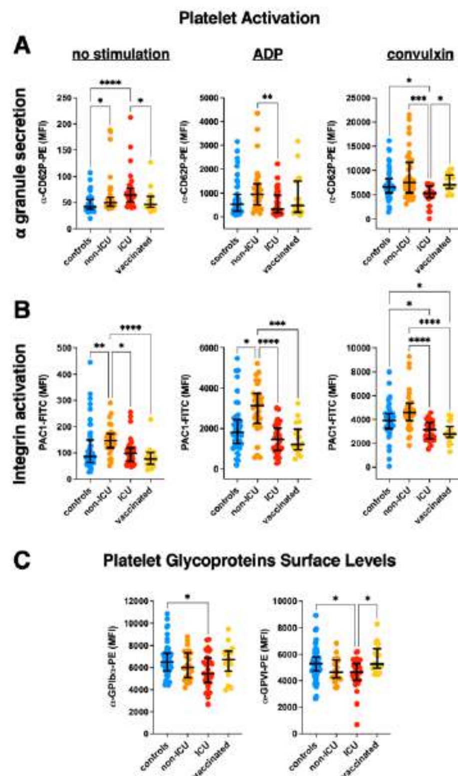


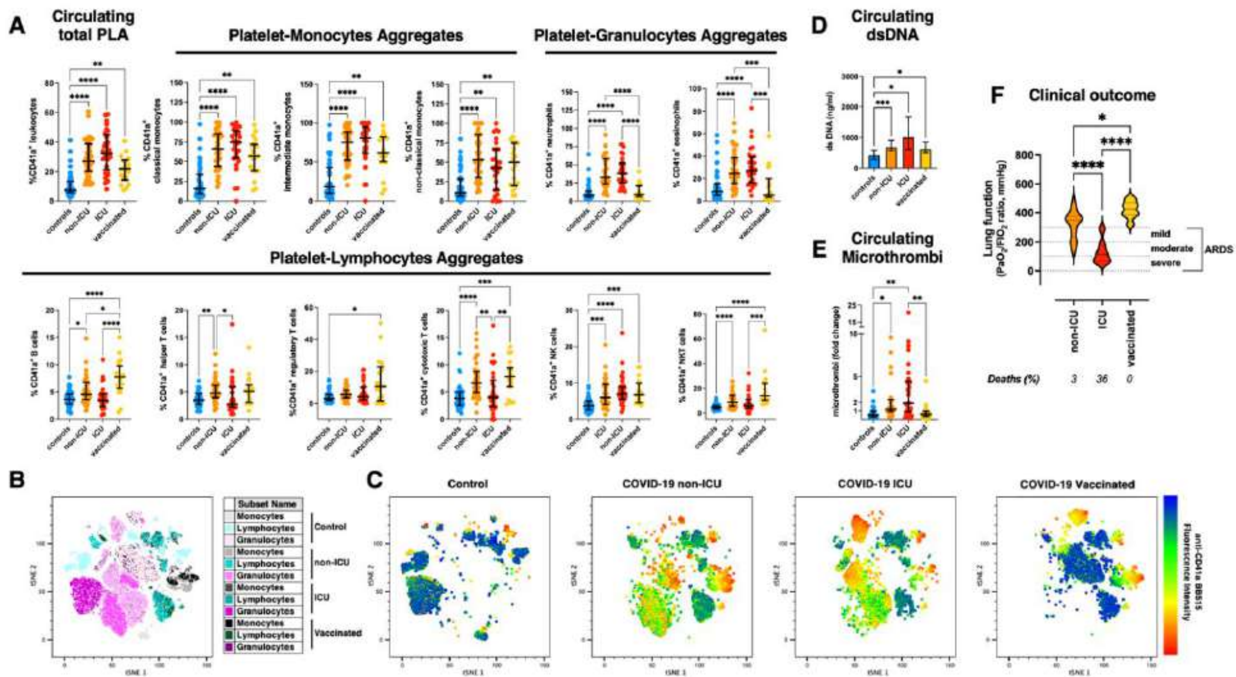
Figure 1. Platelet phenotype in COVID-19

In non-stimulated conditions, circulating platelets of unvaccinated COVID-19 patients displayed high

levels, increasing with severity, of surface P-selectin (CD62P), a marker of α-granule secretion (**Figure 1A**). Activation of the major integrin receptor αIIbβ3 was increased in non-ICU patients, but not in ICU patients (**Figure 1B**). Upon stimulation, platelets from uncomplicated COVID-19 activated significantly more than controls in response to either weak (ADP) or strong (convulxin) agonists, confirming that SARS-CoV-2 infection is associated to a hyperactive platelet phenotype as shown previously (Manne et al., Blood 2020). Conversely, platelets from ICU patients failed to induce P-selectin exposure and integrin activation upon stimulation. Moreover, ICU patients displayed 15% less glycoprotein (GP)Ibα and GPVI on the platelet surface (**Figure 1C**). While P-selectin exposure supports platelet binding to active endothelial and immune cells, integrin αIIbβ3 inactivation (Mattheij et al., JBC 2013) and glycoprotein shedding (Baaten et al., Blood Adv 2018) enhance binding of coagulation factors to the platelet surface. Indeed, simple linear regression analysis identified negative correlations between the plasmatic D-dimer concentrations, the surface expression of GPIbα ($R2=0.13$, $p<0.05$) and GPVI ($R2=0.23$, $p<0.0001$) and the agonist-induced αIIbβ3 activation ($R2=0.2$, $p<0.01$). Thus, high P-selectin exposure, low agonist responsiveness and glycoprotein shedding may be features of super-activated platelets that rewire to minimize adhesion but eventually shift toward a proinflammatory and pro-coagulant phenotype. We hypothesize that this phenotype, very similar to the phenotype of platelets from trauma patients (Vulliamy et al., Blood Adv 2020), is driven by GPIbα and GPVI signalling since ectodomain shedding of these glycoproteins is stimulated by engagement of their respective ligands, Von Willebrand Factor (VWF) (Deng et al., Nat Comm 2016) and fibrin (Montague et al., JTH 2020), which are elevated in severe COVID-19 plasma because of systemic endothelial activation (Mancini et al., JTH 2021) and dysregulation of the coagulation and fibrinolytic pathways (Conway et al, Nat Rev Immunol 2022). Interestingly vaccinated COVID-19 patients had a platelet phenotype and activation pattern comparable to negative controls and did not display platelet hyperactivation nor glycoprotein shedding.

To study if the different functional and adhesive properties of platelets of the four patient groups may influence their ability to interact with circulating immune cells and potentially trigger thromboinflammatory complications, we stained fresh whole blood with a combination of fluorochrome-tagged antibodies that identify platelets bound to 11 leukocyte subsets (Flego et al., JTH 2022). We observed that SARS-CoV-2-positive patients had elevated levels of platelet-leukocyte aggregates independently of their vaccination status, but the frequency of platelet aggregates with specific leukocyte subsets changed across different groups (**Figure 2A**).

Figure 2. Circulating platelet-leukocyte and platelet-platelet in COVID-19

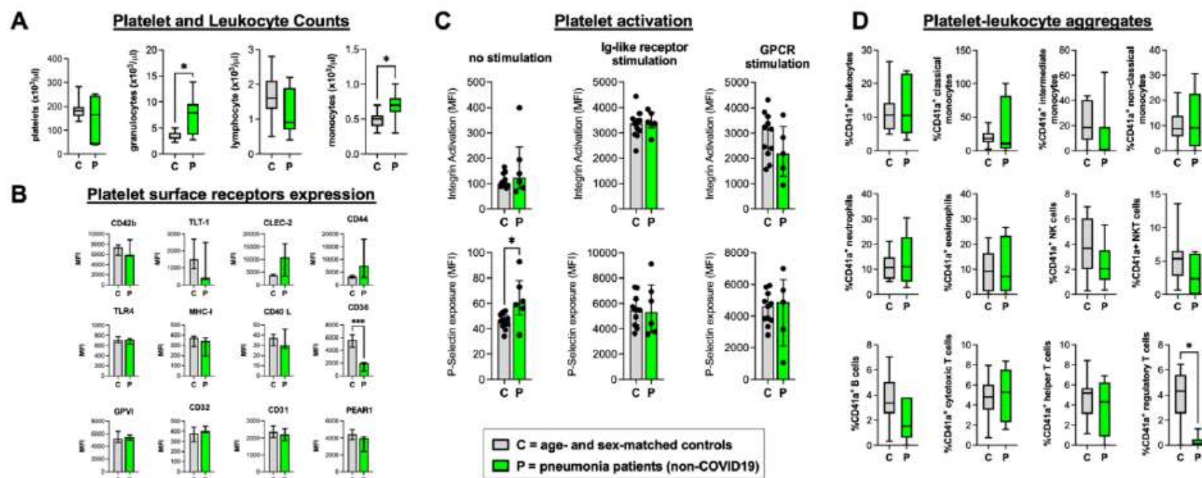


Among unvaccinated COVID-19 patients, aggregates with innate immune cells accounted for most of the interactions, with most patients displaying 70% of monocytes and 40% of neutrophils bound to platelets. Conversely the binding of platelets to lymphocytes, specifically with B-cells, helper and cytotoxic T-cells and natural killer (NK)T-cells, was significantly increased in non-ICU patients but dropped in ICU patients. This binding profile suggests that platelet adhesion to innate immune cells relies more on P-selectin that increases with severity (Figure 1A), while platelet interactions with adaptive immune cells depend more on the ability to activate integrins in response to stimuli. Thus, it fails in critical patients that have exhausted lymphocytes (Kusnadi et al., Sci Immunol 2021) and hypo-adhesive platelets.

Preliminary analysis of our data on non-COVID-19 pneumonia patients (n=6) indicates that platelets, like in COVID-19, display a distinct surface receptor profile (**Figure 3B**)

and increased P-selectin exposure (**Figure 3C**) compared to age-matched controls. However, in the peripheral blood of non-COVID-19 pneumonia we detect elevated levels of aggregates with classical monocytes, but not with granulocytes or with non-classical and intermediate aggregates. Moreover, platelet complexes with regulatory T cells are almost undetectable (**Figure 3D**).

Figure 3. Platelet phenotype and platelet-immune crosstalk in non-COVID19 pneuomnia



Vaccinated COVID-19 patients displayed a platelet-leukocyte binding profile that was distinct from all other unvaccinated patients and had two striking features (**Figure 2 A, B, C**). First, in the blood of vaccinated COVID-19 we detected significantly higher levels of circulating platelet-lymphocyte aggregates compared to the other cohorts. Platelets from vaccinated subjects bound most avidly to B-cells, regulatory and cytotoxic T-cells, and NKT-cells. Anecdotally 2 of the 3 vaccinated patients that were admitted to the ICU had very low platelet-B-cell aggregates, suggesting that their clinical conditions may have worsened because they had failed to evoke an effective adaptive response despite the vaccination. Indeed, delayed production of neutralizing antibodies has been shown to correlate with a worse clinical progression and mortality in COVID-19 (Lucas et al., Nat Med 2021).

Second, in vaccinated COVID-19 we observed a complete normalization of the frequency of platelet-neutrophil and platelet-eosinophil aggregates, while platelet-monocyte aggregates remained high. Accordingly, we found that plasmatic cell-free double stranded DNA (dsDNA), that is not only a marker of tissue damage but also a surrogate marker of neutrophil extracellular traps (NETs), was higher in unvaccinated compared to vaccinated COVID-19 patients (**Figure 2D**).

By further analysing the flow cytometry data on platelet-leukocyte aggregates we detected CD41a+ events that were larger than regular platelets but did not bind leukocytes. These events, that are most likely microthrombi, were significantly higher among unvaccinated COVID-19 patients (**Figure 2E**), particularly in the blood of ICU

patients that had acute respiratory distress syndrome (ARDS) (PaO_2/FiO_2 ratio median [interquartile range]: 114 [69-177] mmHg) (**Figure 2F**). High circulating microthrombi and low αIIbβ3 activation in ICU patients are not conflicting observations since ultra-large VWF (Chauhan et al., JTH 2007) and fibrin (Montague et al., JTH 2020) support the formation of microthrombi in a αIIbβ3-independent manner through agglutination. Despite the old age and the infection, the vaccinated subjects did not display circulating microthrombi and did not experience pulmonary complications (PaO_2/FiO_2 ratio: 424 [386-470] mmHg) (**Figure 2F**). Thus, we speculate that vaccinated subjects by evoking a faster and stronger adaptive response to the virus, are protected from the systemic response that drives aberrant platelet activation (**Figure 1**) and formation of pathological platelet-platelet and platelet-neutrophil aggregates (**Figure 2**) that cause the occlusion of the pulmonary microcirculation and exacerbate the severity of COVID-19.

Intrigued by the detection of high levels of platelet complexes with adaptive immune cells in vaccinated COVID-19 patients, we investigated if vaccinations induced platelet-lymphocyte interactions also in healthy subjects and if these aggregates correlated with the vaccine response. In a first study (Flego et al., JTH 2022) we evaluated the platelet response of a cohort of 11 young healthy adults who were immunized with the BNT162b2 mRNA-based vaccine. We found that, upon vaccination, platelets undergo a more rapid turnover and functional reprogramming and that study participants who developed a faster humoral response had more circulating platelet-leukocyte aggregates. Specifically, in the fast, but not in the slow, vaccine responders we could detect elevated platelet-B cell aggregates as early as 3 days after the first dose of the vaccine. In a second study (Lombardi et al., JTH 2023), we compared the effect of the mRNA-based vaccine BNT162b2 (n=15) and of the adenovirus-based vaccine AZD1222 (n=25) on the platelet and the immune response of young healthy adults. We observed that the adenovirus-based vaccine, the less immunogenic of the two, evokes, shortly after the injection, an acute rise of IFN-γ and other inflammatory cytokines that overlaps in time with an increased activation of platelets and granulocytes, increased frequency of platelet-granulocyte aggregates and a transient drop of the platelet and granulocyte count in the peripheral circulation. The mRNA-based vaccine is associated with a gradual increase of IL-1β, the expansion of regulatory T cells and an increased interaction of platelets with adaptive immune cells. A faster response to both types of vaccines is associated with the formation of platelet aggregates with B cells, primarily with marginal zone-like B cells, a subtype geared to respond rapidly to blood-borne pathogens and to bridge the temporal gap between innate and adaptive immunity.

Collectively our data shows that platelets respond to both infectious and sterile inflammatory stimuli changing phenotype and interacting with innate and adaptive immune cells. Moreover, it provides preliminary evidence that platelets participate in the early immune pathways that facilitate the onset of a rapid humoral response and advocate for the importance of vaccination in preventing thromboinflammatory complications.

In future studies we will continue to study the pathological interplay between platelets and immune cells that give rise to thromboinflammatory complications in infectious disease and in parallel we will further investigate if and how platelets modulate adaptive immunity after vaccinations and in disease.

Publications

Lombardi L, Maiorca F, Marrapodi R, Sabetta A, Scafa N, Pallucci D, Miglionico M, Romiti GF, Corica B, Piconese S, Polimeni A, Pulcinelli F, Cangemi R, Visentini M, Basili S, Stefanini L. ***Distinct platelet crosstalk with adaptive and innate immune cells after adenoviral and mRNA vaccination against SARS-CoV-2.*** J Thromb Haemost. 2023. Epub ahead of print. IF: 16.041

Research Group

2 Researchers: **Francesca Maiorca, Ramona Marrapodi, Annamaria Sabetta**
2 PhD students: **Davide Pallucci, Ludovica Lombardi**

Collaborations

Ivan Zanoni, Harvard Medical School
Judith Cosemans, Mastricht University
Ilaria Canobbio, University of Pavia
Silvia Piconese, Sapienza University
Stefania Basili, Sapienza University
Mary Anna Venneri, Sapienza University

Targeting and hijacking the binding between PDZ domains and the Envelope protein from SARS- CoV and SARS-CoV-2

TARGETING AND HIJACKING THE BINDING BETWEEN PDZ DOMAINS AND THE ENVELOPE PROTEIN FROM SARS-CoV AND SARS-CoV-2

ANGELO TOTO

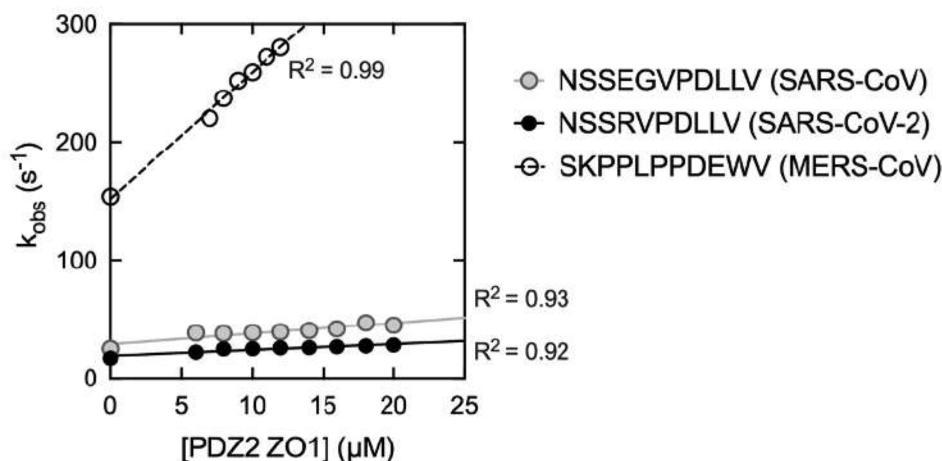
RESEARCH AREA: Infectious agents and associated diseases

Department of Biochemical Sciences “A.Rossi Fanelli”
angelo.toto@uniroma1.it

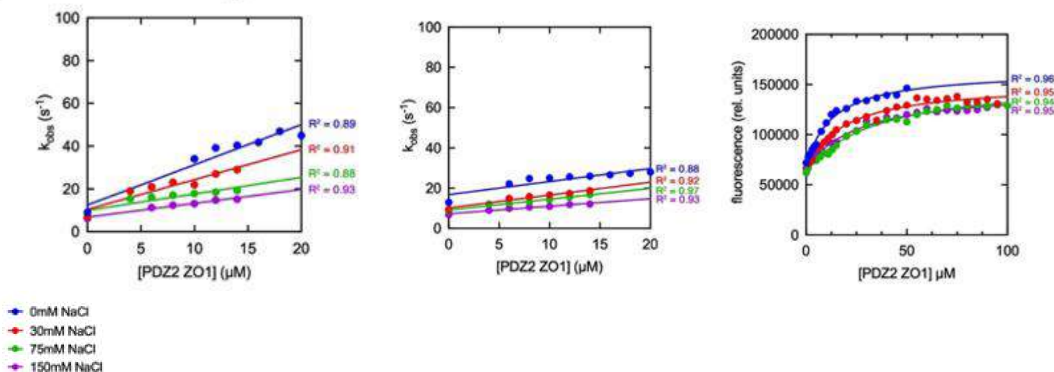
Coronaviruses (CoV) are enveloped viruses that can infect humans at level of the respiratory system, ranging from infections of the upper respiratory tract, resembling the common cold, to the lower respiratory tract causing bronchitis and pneumonia. The E protein is the shortest and most enigmatic of the four structural proteins. It is an integral membrane protein in which three domains can be identified: a hydrophobic transmembrane domain (TMD), responsible of the formation of an alpha-helical structure that undergoes oligomerization and subsequent constitution of an ion channel in the membrane, flanked by a short hydrophilic N-terminal domain (NTD) and the largest hydrophilic C-terminal domain (CTD). The CTD of E proteins of SARS-CoV, SARS-CoV-2 and MERS-CoV (three members of the Coronaviruses family) has been established to display, at its C-terminus, a PDZ-binding motif (PBM), that is a consensus sequence recognized and bound by PDZ domains. The PDZ2 domain of ZO1 is known to interact with the Coronaviruses Envelope proteins, however the molecular details of such interaction have not been established. We directly measured, through Fluorescence Resonance Energy Transfer and Stopped-Flow methodology, the binding kinetics of the PDZ2 domain of ZO1 with peptides mimicking the C-terminal portion of the Envelope protein from SARS-CoV, SARS-CoV-2 and MERS-CoV in different ionic strength conditions.

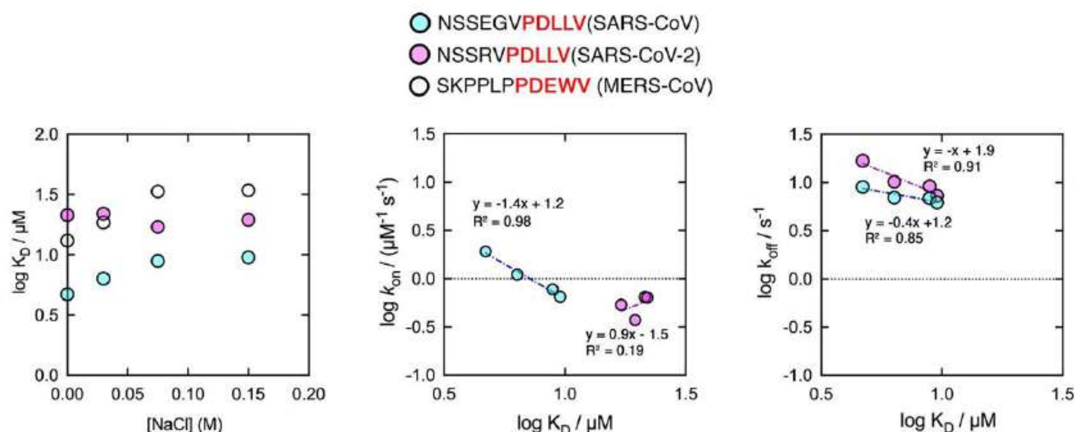
To spectroscopically monitor the kinetics of the binding between PDZ2 of ZO1 and peptides mimicking Envelope protein we resorted to measure them through Fluorescence Resonance Energy Transfer (FRET). We engineered a pseudo wild-type variant of PDZ2, by mutating the F residue in position 207 to W (F207W) to act as a donor group, the acceptor being a dansyl group covalently linked to the N-terminus of the peptides. Kinetic binding experiments were performed with a SX-18 Stopped-Flow apparatus (Applied Photophysics) by rapidly mixing a constant concentration of peptide mimicking the E proteins versus increasing concentrations of PDZ2 Y207W.

The analysis of kinetic binding experiments allowed us to calculate the microscopic association (k_{on}) and dissociation (k_{off}) rate constants of the binding reaction.



Data reported in figure above highlight the ability of the Envelope protein from MERS-CoV to reach a more rapid equilibrium with the PDZ2 of ZO1 compared to the SARS-CoV and SARS-CoV-2 Envelope proteins, and a higher affinity for PDZ2 by a factor of ~2. Interestingly, the k_{on} measured for MERS-CoV E peptide (10.7 ± 0.3 μM^{-1} s^{-1}) is one order of magnitude higher compared to the k_{on} of SARS-CoV (1.9 ± 0.2 μM^{-1} s^{-1}) and two orders of magnitude higher compared to the k_{on} of SARS-CoV-2 (0.6 ± 0.1 μM^{-1} s^{-1}). This result suggested the formation of more favorable electrostatic interactions in the case of MERS-CoV E peptide, which were investigated by performing kinetic binding experiments at different concentrations of NaCl added to the buffer, i.e., increasing the ionic strength. For the MERS-CoV E peptide the addition of salt to the buffer caused a dramatic decrease of kinetic amplitude that forced us to monitor the binding reaction through equilibrium binding experiments, while we could monitor SARS-CoV and SARS-CoV-2 peptides through stopped-flow.





The analysis of kinetic parameters put in evidence different contributions of electrostatics in the recognition and complex formation events for the three peptides. This analysis, comparing the PDZ binding motif presented by the three peptides employed in this study and under the light of structural data available about the PDZ2 domain of ZO1 suggests the formation of different transient electrostatic interactions that may occur outside of the PDZ domain binding pocket.

A manuscript is currently under the second peer revision and awaiting for Editor's Decision in the journal Scientific Reports – Nature Publishing Group.

Publications

- Malagrinò F, Pennacchietti V, Santorelli D, Pagano L, Nardella C, Diop A, Toto A, Gianni S. On the Effects of Disordered Tails, Supertertiary Structure and Quinary Interactions on the Folding and Function of Protein Domains. Biomolecules. 2022 Jan 26;12(2):209 IF: 6.064
- Coluccia A, Bufano M, La Regina G, Puxeddu M, Toto A, Paone A, Bouzidi A, Musto G, Badolati N, Orlando V, Biagioni S, Masci D, Cantatore C, Cirilli R, Cutruzzolà F, Gianni S, Stornaiuolo M, Silvestri R. Anticancer Activity of (S)-5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(1-oxo-1-((pyridin-4-ylmethyl)amino)propan-2-yl)-1H-indole-2-carboxamide (RS4690), a New Dishevelled 1 Inhibitor. Cancers (Basel). 2022 Mar 7;14(5):1358 IF: 6.575
- Toto A, Malagrinò F, Nardella C, Pennacchietti V, Pagano L, Santorelli D, Diop A, Gianni S. Characterization of early and late transition states of the folding pathway of a SH2 domain. Protein Sci. 2022 Jun;31(6):e4332 IF: 6.993
- Nardella C, Toto A, Santorelli D, Pagano L, Diop A, Pennacchietti V, Pietrangeli P, Marcocci L, Malagrinò F, Gianni S. Folding and Binding Mechanisms of the SH2 Domain from Crkl. Biomolecules. 2022 Jul 22;12(8):1014 IF: 6.064

- Malagrinò F, Fusco G, Pennacchietti V, Toto A, Nardella C, Pagano L, de Simone A, Gianni S. Cryptic binding properties of a transient folding intermediate in a PDZ tandem repeat. Protein Sci. 2022 Sep;31(9):e4396 IF: 6.993
- Pagano L, Pennacchietti V, Diop A, Santorelli D, Pietrangeli P, Marcocci L, Nardella C, Malagrinò F, Toto A, Gianni S. Exploring the effect of tethered domains on the folding of Grb2 protein. Arch Biochem Biophys. 2022 Nov 30;731:109444 IF: 4.114
- Diop A, Santorelli D, Malagrinò F, Nardella C, Pennacchietti V, Pagano L, Marcocci L, Pietrangeli P, Gianni S, Toto A. SH2 Domains: Folding, Binding and Therapeutical Approaches. Int J Mol Sci. 2022 Dec 15;23(24):15944 IF: 6.208

Research Group

Valeria Pennacchietti PhD student

Collaborations

Prof. Alessandro Arcovito, Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Roma, Italy

COLLABORATIONS WITHIN THE INTERNATIONAL NETWORK OF INSTITUTES PASTEUR

Funded by Istitut Pasteur Paris:

PTR - PROGRAMMES TRASVERSAUX DES RECHERCHE
ACIP - PROJETS TRASVERSAUX DE RECHERCE

PTR 2022

TUNING THE ADAR1 RNA EDITING ENZYME TO BOOST TYPE I IFN AND NK CELL INNATE IMMUNE RESPONSES IN THE MODEL OF HPV-TRANSFORMED CELLS

Cristina Cerboni[1], Jean-Pierre Vartanian[2], Marcel Hollenstein[2]
1. IP Rome, 2. IP Paris

The hypothesis driving this project is that in high-risk (hr) HPV-transformed epithelia, the RNA editing enzyme ADAR1 could play a role in hrHPV-driven tumorigenesis, by its ability to dampen the activation of IFN-I pathways and innate immune responses. Therefore, we are investigating for the first time the interplay between ADAR1, IFN-I, and innate lymphocytes (i.e., NK cells and innate lymphoid cells, ILCs).
The project is divided in 3 work packages (WP), each one with a different leader.

In WP1 (leader: Jean-Pierre Vartanian), the aim is to analyse the interaction partners of ADAR1 that could influence the degree of editing *(task 1)*. We want to demonstrate that the burden of mutations following multiple rounds of ADAR1 induction during chronic inflammation will lead to tumorigenesis *(task 2)*. Validation of the ADAR1 mutated phenotype will be performed in patients to correlate ADAR1 expression and editing activities with tumorigenesis *(task 3)*.
For *task 1,* we we are analyzing proteins interacting with p150 ADAR1 (full-length or isolated domains) in a yeast two-hybrid system. A cDNA library derived from the A549 cell line was introduced by transformation into a suitable strain of yeast and used to screen for ADAR1 interactors. We have now detected about 100 interactors to be sequenced.
In a parallel set of experiments, we developed a cell-based system for conditional expression of human ADAR1 to show that it edits by sequential mutational waves (*task 2)*. We obtained the constructs and HeLa cells will be now stably transduced with a doxycycline inducible GFP-ADAR1 and GFP-ADAR1* (catalytic mutant) lentiviral vector. Each ADAR1-expressing selected single cell will be expanded without induction and then subjected to 10 rounds of ADAR1 and ADAR1* induction and recovery. We also plan to make RNAseq analysis from cervical cancer (CC) cells, silenced or not for ADAR1, as well in fresh CC biopsies, in search for A-to-I mutations on ADAR1-edited sites *(task 3)*.

In WP2 (leader: Cristina Cerboni), the aim is to explore how the IFN-I pathway can be manipulated through ADAR1 to promote inflammation and infiltration of innate lymphocytes in CC.
We are currently dissecting the *intrinsic effects* that ADAR1 silencing has on HPV-transformed CC cells (i.e., SiHa and CaSki cell lines), focusing on the IFN-I signaling

pathway, pro-inflammatory cytokine production, and cell survival/proliferation of silenced (KO) cells. Indeed, data collected so far clearly show that CC cell lines express boh ADAR1 isoforms (Fig. 1A), and that ADAR1 KO cells have a decreased proliferative capacity, particularly after treatment with IFN-beta (Fig. 1B). Innate immunity pathways are also activated in KO cells, with increased levels of IFN-alfa1, IFN-beta1, IFN-lamda1 and of other immune response genes (Fig. 1C), as well as of STAT1 and PKR phosphorylation (Thr446/Thr451) (Fig. 1D) (similar results for CaSki cells; not shown) *(Task 1).*
To correlate these findings with the dysregulation of NK cell effector functions against hrHPV-transformed cells (*extrinsic effects, Task 2),* supernatants (SNs) from KO SiHa cells were used to verify the release of pro-inflammatory cytokines/chemokines able to activate NK cells. Data show that NK cells incubated with such SNs increase their proliferation (Fig. 2A; similar data for CaSki cells, not shown), as well as their degranulation activity (CD107a+) against both K562 and the less sensitive target SiHa (Fig. 2B-C). These results suggest that ADAR1 silencing in tumor cells may (re)activate innate immune responses. We are currently aiming at identifying cytokines/chemokines modulated by ADAR1 inhibition and released in the supernatants, able to enhance NK cell activities.
For *Task 3,* by *ex vivo* and *in vivo* analysis, we are collecting and analyzing fresh biopsies and paraffin-embedded tissue sections of cervical cancers for the presence of innate lymphocytes (NK/ILCs) infiltrating the tumor. Preliminary results demonstrate the presence of a cervix-innate infiltrate, with the Lin-CD45+CD7+ cells including both NK and NCR+ILC3 (Fig. 3A). The correlation between ADAR1 expression and CD56+ (NCAM+) NK cells is under investigation also by IHC (Fig. 3B), with the aim of understanding an ADAR1-dependent reshaping of NK cell/ILC innate immune responses and/or impact on inflammation-driven immune responses. We also plan to extract RNA from the same biopsies and perform RNAseq and analysis of A-to-I mutations.
Finally, in relation to the development of scaffold-based 3D skin-like structures *(Task 4)*, we are testing different compositions of matrices (with collagen, fibronectin and/or laminin), as well as a new approach based on organotypic cell cultures. These 3D cultures could be used to study a three-dimensional relationship between CC cells and innate leukocytes, as well as to validate aptamers developed by Dr. M. Hollenstein (see also WP3).

In WP3 (leader: Marcel Hollenstein), the aim is to identify aptamers that specifically bind to hrHPV-transformed cells, and then convert them to a system capable of delivering an siRNA oligonucleotide capable of silencing the expression of ADAR1 and thus to restore IFN-I production. Ultimately, this would cause anti-tumor immunity and block of cell proliferation in hrHPV-transformed cells. In addition, since ADAR1 is overexpressed in various forms of cancer, we wish to exploit this feat to develop an alternative therapeutic approach yet based on aptamers. Indeed, we aim at harnessing the A-to-I editing property of endogenous ADAR1 to correct known cancer-related

mutations (such as E542K/E545K on PIK3CA) by conjugating the afore-mentioned aptamers to AIMers, which are short, chemically modified oligonucleotides that elicit A-to-I RNA editing (Monian P et al., Nature Biotechnol, 2022; Chen G et al., Biochemistry 2019). The aptamer part of these conjugates will deliver the AIMers specifically to CC cells and upon cellular internalization, the AIMers will make use of endogenous ADAR1 to specifically induce mutations in selected RNAs.

We are currently expressing the His-tagged protein ADAR1 to initiate SELEX against this protein, under typical conditions used in the laboratory (see e.g., Cheung YW et al., PNAS 2020) using standard DNA chemistry *(Task 1)*. We have also prepared a degenerate library that will be used after purification of the protein *(Task 1)*. Lastly, we have started to work on the synthesis of chemically modified RNA molecules *(Task 2)*. Regarding AIMers, we have already synthesized various sugar-modified RNA oligonucleotides. Once ADAR1 protein will be expressedand purified, we will evaluate the editing capacity of the newly synthesized RNA oligonucleotides on a proof-of-principle RNA target sequence *(Task 2 revised)*. Briefly, we will incubate target RNA with ADAR1 and the chemically modified oligonucleotides and evaluate RNA editing *in vitro* (see Stafforst et al., Angew Chem Int Ed Eng, 2012). If successful, the RNA oligonucleotides will be transfected into cancer cells and RNA editing will be evaluated *in vivo*.

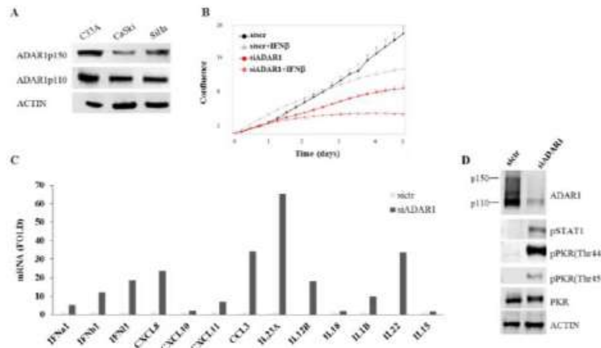


Figure 1. Analysis of ADAR1 expression in cervical cancer cell lines and impact on IFN-I pathway. A) Immunoblot analysis showing p110 and p150 ADAR1 expression in CC cell lines. B) Proliferation of siscr and siADAR1 (30 nM) SiHa cells, treated or not with IFNbeta (1000 IU/ml), was monitored over 5 days by the Incucyte® live-cell analysis system. C) qRT-PCR showing *IFNs* and ISGs expression in sictr and siADAR1 SiHa cells, 72 hrs following ADAR1 silencing. *GAPDH* was used as a control. D) Immunoblot showing increased STAT1 and PKR phosphorylation in siADAR1 SiHa cells compared to sictr, 72 hrs post-siRNA transfection.

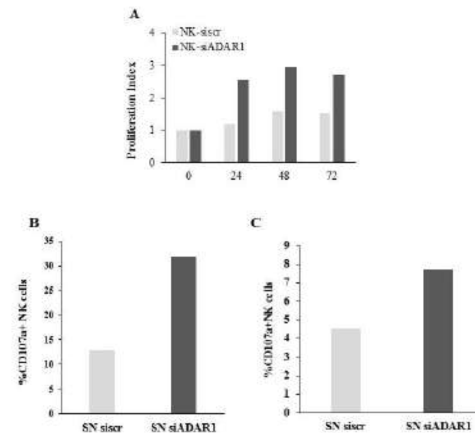
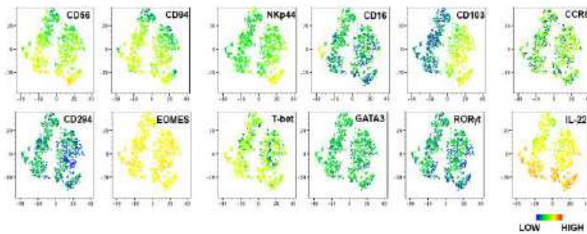


Figure 2. Impact of ADAR1 modification on NK cell phenotype. A) Conditioned supernatants (SNs) from sictr and

siADAR1 SiHa cells were harvested and incubated for the indicated time points on purified NK cells, obtained from healthy donors. Proliferation was measured by Incucyte® live-cell analysis system and calculated by setting at 1 NK cells at day 0. B) NK cells were incubated O/N with conditioned SNs from sictr or siADAR1 SiHa cells and then used as effectors in a degranulation assay against K562 cells, or against SiHa cells (C), used as targets.



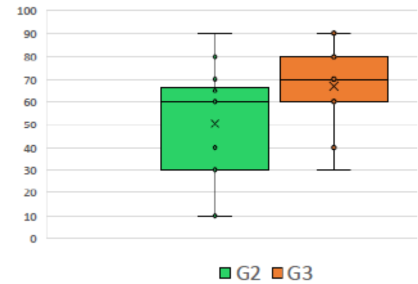


Figure 3. Human innate lymphocytes infiltrating neoplastic cervix. A) t-SNE plot of phenotypic markers on Lin-CD45+CD7+ innate lymphocyte infiltrate in a cervical cancer biopsy. Cells are coloured according to the expression level of the different markers. Results show the presence of NK cells (Lin-CD56+CD16+/-CD94+Eomes+T-bet+CD103-NKp44-IFNgamma+) and of NCR+ILC3 (Lin-CD56+Eomes+T-bet+CD103+NKp44+IL-22+). B) ADAR1 stained sections were divided into high (NCAM>5) and low NK numbers (NCAM<5) (NCAM=CD56) (data not shown). The graph shows that in tumors with low NK numbers (NCAM<5), more aggressive (G3) lesions have a higher cytoplasmic ADAR1 expression.

Publications

1. Tassinari V., Cerboni C., Soriani A. Self or Non-Self? It Is also a Matter of RNA Recognition and Editing by ADAR1. Biology 2002. doi: 10.3390/biology11040568. IF: 5,168.
2. Girone G., Calati F., Lo Cigno I., Salvi V., Tassinari V., Schioppa T., Borgogna C., Lospinoso Severini L., Hiscott J., Cerboni C., Soriani A., Bosisio D., Gariglio M. The RIG-I Agonist M8 Triggers Cell Death and Natural Killer Cell Activation in Human Papillomavirus-Associated Cancer and Potentiates Cisplatin Cytotoxicity. Under second revision, Cancer Immunology Immunotherapy. IF: 6,630.

Research Group

Alessandra Soriani, Associate Professor
Marta Kaciulis, PhD student
Lorenzo Cuollo, PhD student
Stefano Petrai, PhD student

Collaborations

Prof. J.P. Vartanian, Pasteur Institute, Paris
Dr. M. Hollenstein, Pasteur Institute, Paris
Prof. H. Stabile, Sapienza University
Prof. M. Gariglio, University of Piemonte Orientale Medical School
Dr. I. Palaia, Sapienza University Umberto I Hospital
Dr. A. Pernazza, Sapienza University Umberto I Hospital

ACIP 2022

UNDERSTANDING AND HIJACKING THE ONCOGENIC SIGNALLING ADAPTOR PROTEINS CRKL Gab2 AND Frs2

STEFANO GIANNI
RESEARCH AREA: Genetica, biologia e fisiopatologia molecolare-cellulare degli Eucarioti

Receptor tyrosine kinase (RTK) signaling plays key roles in cell physiology and development. Impaired activation of these signaling pathways is critical in the genesis and progression of many types of cancers. RTK signaling pathways are generally activated by growth factor binding to a specific trans-membrane receptor, which activates downstream signaling molecules. These mechanisms require the formation of specific protein complexes mediated by adaptor proteins. Thus, adaptor proteins, while lacking any enzymatic activity provide a critical scaffolding function that facilitates key signaling transduction events and regulates signal specificity and amplification. Among these adaptors, three proteins, namely CRKL, GAB2, and FRS2, represent particularly interesting targets, being recurrently amplified in several types of cancers and essential to cancer cell lines that harbor such amplification. The overexpression of these three proteins is able to transform immortalized human cell lines in in vitro or in vivo models and their knockdown significantly reduces cancer proliferation. Based on these observations, we propose that an effective chemotherapeutic strategy would be that of interfering with CRKL, GAB2 and FRS2 protein-protein interaction network.

To provide a detailed structural characterization of the interactions between CRKL, GAB2 and FRS2 and thier partners, we focused on the interactions between these proteins and p85, Grb2 and SHP2. Therefore we successfully cloned, expressed and purified the N- and C-terminal SH2 domains from p85, the SH3 domain from Grb2 and the N- and C-terminal SH2 domains from SHP2. Subsequently, these constructs were subjected to NMR characterization both in the presence and in the absence of a peptide mimicking GAB2 , which allowed us to assign the structural changes induced by binding. Furthermore, we conducted an extensive biophysical analysis on these proteins domains, which allowed us to describe the mechanism of recognition of these proteins at nearly atomic resolution.

Finally, the research group was also involved in the characterization of the folding of several protein domains, with particular emphasis on the role of intradomain communication in multidomain proteins.

Publications

1) Malagrinò F, Pennacchietti V, Santorelli D, Pagano L, Nardella C, Diop A, Toto A, Gianni S. ***On the Effects of Disordered Tails, Supertertiary Structure and Quinary Interactions on the Folding and Function of Protein Domains*** Biomolecules. 2022 Jan 26;12(2):209. doi: 10.3390/biom12020209 I.F. 6.064

2) Malagrinò F, Diop A, Pagano L, Nardella C, Toto A, Gianni S. ***Unveiling induced folding of intrinsically disordered proteins - Protein engineering, frustration and emerging themes*** Curr Opin Struct Biol . 2022 Feb;72:153-160. doi: 10.1016/j.sbi.2021.11.004. I.F. 7.786

3) Coluccia A, Bufano M, La Regina G, Puxeddu M, Toto A, Paone A, Bouzidi A, Musto G, Badolati N, Orlando V, Biagioni S, Masci D, Cantatore C, Cirilli R, Cutruzzolà F, Gianni S, Stornaiuolo M, Silvestri R. ***Anticancer Activity of (S)-5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)- N-(1-oxo-1-((pyridin-4-ylmethyl)amino)propan-2-yl)-1 H-indole-2-carboxamide (RS4690), a New Dishevelled 1 Inhibitor*** Cancers (Basel). 2022 Mar 7;14(5):1358. doi: 10.3390/cancers14051358. I.F. 6.575

4) Toto A, Malagrinò F, Nardella C, Pennacchietti V, Pagano L, Santorelli D, Diop A, Gianni S. ***Characterization of early and late transition states of the folding pathway of a SH2 domain*** Protein Sci. 2022 Jun;31(6):e4332. doi: 10.1002/pro.4332.
I.F. 6.993

5) Brunori M, Gianni S. ***An Outlook on the Complexity of Protein Morphogenesis in Health and Disease.*** Front Mol Biosci. 2022 Jun 13;9:909567. doi: 10.3389/fmolb.2022.909567. I.F. 6.113

6) Nardella C, Toto A, Santorelli D, Pagano L, Diop A, Pennacchietti V, Pietrangeli P, Marcocci L, Malagrinò F, Gianni S. ***Folding and Binding Mechanisms of the SH2 Domain from Crkl.*** Biomolecules. 2022 Jul 22;12(8):1014. doi: 10.3390/biom12081014.
I.F. 6.064

7) Malagrinò F, Fusco G, Pennacchietti V, Toto A, Nardella C, Pagano L, de Simone A, Gianni S. ***Cryptic binding properties of a transient folding intermediate in a PDZ tandem repeat.*** Protein Sci. 2022 Sep;31(9):e4396. doi: 10.1002/pro.4396.
I.F. 6.993

8) Gautier C, Gianni S. A short structural extension dictates the early stages of folding of a PDZ domain. Biochim Biophys Acta Proteins Proteom. 2022 Nov 1;1870(11-12):140852.
I.F. 4.125

9) Pagano L, Pennacchietti V, Diop A, Santorelli D, Pietrangeli P, Marcocci L, Nardella C, Malagrinò F, Toto A, Gianni S. ***Exploring the effect of tethered domains on the folding of Grb2 protein.*** Arch Biochem Biophys. 2022 Nov 30;731:109444. I.F. 4.114

10) Hollås H, Ramirez J, Nominé Y, Kostmann C, Toto A, Gianni S, Travé G, Vedeler A. ***The cooperative folding of annexin A2 relies on a transient nonnative intermediate.*** Biophys J. 2022 Dec 6;121(23):4492-4504. I.F. 4.033

11) Diop A, Santorelli D, Malagrinò F, Nardella C, Pennacchietti V, Pagano L, Marcocci L, Pietrangeli P, Gianni S, Toto A. ***SH2 Domains: Folding, Binding and Therapeutical Approaches.*** Int J Mol Sci. 2022 Dec 15;23(24):15944. I.F. 6.208

Research Group

Angelo Toto Researchers.
Caterina Nardella, Post Doc
Daniele Santorelli, Post Doc
Livia Pagano, PhD student
Awa Diop, PhD student
Valeria Pennacchietti, PhD student

Collaborations (if other than Pasteur)

Per Jemth, Uppsala University

SCIENTIFIC BOARD RESEARCH PROJECTS

INVOLVEMENT OF MULTIDRUG EFFLUX PUMPS IN THE VIRULENCE OF PATHOGENIC E.COLI

BIANCA COLONNA
RESEARCH AREA: Microbiology

Department of Biology and Biotechnologies Charles Darwin
bianca.colonna @uniroma1.it

Efflux pumps represent an important group of transporters found in all organisms. Their importance resides in their ability to extrude a wide range of antibiotics resulting in the emergence of multidrug resistance (MDR) in many bacteria. Besides antibiotics, MDR efflux pumps are capable of excreting a large range of toxic substances including endogenous metabolites, thus facilitating the survival of bacteria under different environmental conditions. This study is part of a larger project aimed at understanding the factors that contribute to virulence in enteropathogenic *E. coli* strains. Indeed, the pathogenicity of a bacterium is a multifactorial process in which several proteins are involved in the different stages of the pathogen's interaction with host target cells. In addition, the bacterium is able to sense the host environment and to trigger a response involving the coordinated activation of virulence genes. In this context, the regulation of many MDR efflux pumps by global regulatory networks, as the Two-Component Systems (TCS), reflects the need of the bacterial cell to fine tune the expression of MDR efflux pumps in response to a specific niche and in coordination with other virulence factors.
In recent years, a significant amount of data on the physiological function and regulatory mechanisms of MDR efflux pumps has been acquired in several bacterial system. This has led to a deeper understanding of the varied biological role of MDR efflux pumps, which are now known to affect, besides drug transport, also virulence, resistance to host defence systems, and biofilm production. In particular, in many cases the extrusion of antibiotics is not the original physiological function of MDR efflux pumps, which should rather be regarded as sophisticated machines contributing to optimize bacterial interactions with other cells. The complexity of the regulatory systems underlying the expression of MDR efflux pumps is in line with the need to rapidly and co-ordinately activate the expression of efflux pump genes in response to a broad range of substrates and environmental signals. In the last years our group has analysed the expression of MDR efflux pumps during the intracellular life of *Shigella flexneri* and of Adherent Invasive *E. coli* (AIEC), two groups of pathogenic bacteria sharing the ability to invade macrophages and epithelial cells (Pasqua et al., 2019, Fanelli et al., 2020). *S. flexneri* is responsible for human bacillary dysentery, while AIEC is associated with Crohn's disease. Despite the capability to invade the same host cells, AIEC and *Shigella* exhibit different intracellular survival strategies. The different behavior of AIEC and *Shigella* within host cells has prompted us to investigate the expression of the MDR EPs of

clinical strain LF82, considered as an AIEC prototype, during the invasion of epithelial cells and macrophages. The results we have obtained indicate that within these environments bacteria display a strong induction of several EPs, some of which are host-cell specific. As the extensive replication of LF82 in macrophage phagolysosomes is a critical step for the intracellular survival of the pathogen, we have focused on EPs highly expressed only within macrophages and have found that MdtEF, a MDR EP belonging to the RND family, significantly contributes to bacterial fitness in this environment. More recently, we have observed that another MDR efflux pump belonging to the RND family, AcrAB, is required for the full expression of pathogenicity (Fanelli et al., 2023). AcrAB is primarily responsible for resistance to a broad class of antibiotics and has been described to be relevant for the virulence phenotype of several bacterial pathogens. The results from infections of Caco-2 intestinal epithelial cells and of THP-1-derived macrophages we have obtained suggest that the loss of AcrAB components greatly affects bacterial survival in the macrophage environment, while viability in epithelial cells is only very marginally touched. The requirement of AcrB transporter activity for intramacrophage survival was confirmed by loss of function approaches using either an EP inhibitor or a LF82 strain expressing an unfunctional AcrB form in the infection experiments.
Overall, the results we have obtained clearly underline how MDR efflux pumps are expressed in response to cell specific stimuli and stress the relevance of some MDR pumps in favouring the bacterial survival within host cells (Pasqua et al., 2021). Moreover, our data highlight how the expression of MDR pumps is submitted to a complex regulation by several factors, including Two-component Systems (TCS). The TCS play an important role in recognizing environmental signals through a sensor, i.e. a membrane kinase able to autophosphorylate in response to specific stimuli. The sensor then transfers the phosphate group to a regulator, which, in its phosphorylated state, generally activates several genes. TCSs are emerging as crucial regulators of the virulence phenotype in an increasing number of life-threating bacterial pathogens (Pasqua et al., 2022). In this context, understanding the mechanisms of TCS-mediated virulence regulation in pathogenic *E.coli*, constitutes a fascinating and complex challenge to better decipher the strategies adopted by these pathogens to respond to host environment. Morevoer, considering the relevance of TCSs in the expression of virulence in pathogenic bacteria, the identification of drugs that inhibit TCS function may represent a promising approach to combat bacterial infections.

Publications

Pasqua M, Coluccia M, Eguchi Y, Okajima T, Grossi M, Prosseda G, Utsumi R, Colonna B. ***Roles of Two-Component Signal Transduction Systems in Shigella Virulence***. Biomolecules. 2022 12(9):1321. IF 6.064

Research Group

Gianni Prosseda, Associate Professor
Milena Grossi Researcher;
Martina Pasqua post-doc
Giulia Fanelli post-doc
Rita Trirocco PhD student
Marco Coluccia PhD student

Collaborations

Frederic Barras, Institut Pasteur, Paris (FR)
Sarah Dubrac ,Institut Pasteur, Paris, (FR)
Ryutaro Utsumi, Sanken Institute, Osaka University, Osaka (JP)

METABOLIC REPROGRAMMING IN PHYSIOLOGY AND PATHOLOGY

FRANCESCA CUTRUZZOLÀ
RESEARCH AREA: Genetics, biology and pathophysiology of eukaryotes

Department of Biochemical Sciences "A.Rossi Fanelli"

francesca.cutruzzola@uniroma1.it

Metabolic reprogramming is a well-known process occurring in cancer as well as in other cell types, like immune cells, proliferating epithelia or during cell differentiation.
Our project aims at understanding how reshaping of cellular metabolism is linked to uncontrolled cell growth and maintenance of the proliferation potential in cancer as well as in other pathological states of genetic and sporadic origin.
We have focused our attention on serine-glycine one-carbon metabolism (SGOC), a crucial metabolic pathway that fuels the folate and methionine cycles thereby providing cells with the building blocks, as well as the reducing power, necessary to maintain high rates of proliferation. In several cases, an increased activity of the key SGOC enzyme *serine hydroxymethyltransferase (SHMT),* responsible for reversible conversion of Ser and tetrahydrofolate (THF) into Gly and 5,10-methylene-THF, is observed. *All available data suggest that SHMT is a remarkably complex enzyme,* for several reasons**.**
SHMT1 is a critical part of the dTMP Synthesis Complex (dTMP-SC) including thymidylate synthase (TYMS) and dihydrofolate reductase (DHFR). Changes in SHMT1 expression directly impact *de novo* dTMP synthesis by affecting dTMP-SC assembly and its inactivation causes uracil misincorporation and genomic instability. We have recently characterized the intracellular dynamics of the complex in cancer cells by an in situ proximity ligation assay, showing that it is also detected in the cytoplasm (Spizzichino et al., 2022, cover image on the journal). This result indicates that the role of the thymidylate synthesis complex assembly may go beyond dTMP synthesis. We have also successfully assembled the dTMP synthesis complex in vitro, employing tetrameric SHMT1 and a bifunctional chimeric enzyme comprising human thymidylate synthase and dihydrofolate reductase. We show that the SHMT1 tetrameric state is required for efficient complex assembly, indicating that this aggregation state is evolutionarily selected in eukaryotes to optimize protein-protein interactions.
We have completed the determination by cryo-EM of the three-dimensional structure of SHMT1 both alone and in complex with RNA, showing how RNA can control the enzyme's activity (Spizzichino et al, submitted).
We have also pursued our analysis of the effect of SHMT on cell migration, suggesting that serine, glycine and glutamate are the most relevant amino acids both in vitro and in mouse models (manuscript in preparation), to verify the therapeutic potential of selected molecules in controlling metastasis. The study of metabolic remodeling was also carried

out in collaboration on other settings, such as antiviral response (Zevini et al., 2022), cancer (Coluccia et al., 2022) and biofilm formation (Scribani Rossi et al, 2022).

Publications

Zevini A, Palermo E, Di Carlo D, Alexandridi M, Rinaldo S, Paone A, Cutruzzolà F, Etna MP, Coccia EM, Olagnier D, Hiscott J ***Inhibition of Glycolysis Impairs Retinoic Acid-Inducible Gene I-Mediated Antiviral Responses in Primary Human Dendritic Cells.*** Front Cell Infect Microbiol. 2022 Jul 18;12:910864. doi: 10.3389/fcimb.2022.910864. IF 6,073

Scribani Rossi C, Barrientos-Moreno L, Paone A, Cutruzzolà F, Paiardini A, Espinosa-Urgel M, Rinaldo S. **Nutrient Sensing and Biofilm Modulation: The Example of L-arginine in *Pseudomonas***. Int J Mol Sci. 2022 23(8):4386. doi: 10.3390/ijms23084386. IF 6,208

Coluccia A, Bufano M, La Regina G, Puxeddu M, Toto A, Paone A, Bouzidi A, Musto G, Badolati N, Orlando V, Biagioni S, Masci D, Cantatore C, Cirilli R, Cutruzzolà F, Gianni S, Stornaiuolo M, Silvestri R. ***Anticancer Activity of (S)-5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(1-oxo-1-((pyridin-4-ylmethyl)amino)propan-2-yl)-1H-indole-2-carboxamide (RS4690), a New Dishevelled 1 Inhibitor.*** Cancers (Basel). 2022, 14(5):1358. doi: 10.3390/cancers14051358. IF: 6,639

Spizzichino S, Boi D, Boumis G, Lucchi R, Liberati FR, Capelli D, Montanari R, Pochetti G, Piacentini R, Parisi G, Paone A, Rinaldo S, Contestabile R, Tramonti A, Paiardini A, Giardina G, Cutruzzolà F. ***Cytosolic localization and in vitro assembly of human de novo thymidylate synthesis complex.*** FEBS J. 2022 Mar;289(6):1625-1649. IF 5,542.

Research Group

Sharon Spizzichino
Post-doctoral fellow
Francesca Liberati PhD student
Federica di Fonzo PhD Student
Agnese Riva PhD Student

Collaborations

Gian Gaetano Tartaglia, Sapienza University of Rome (Italy)
Javier A. Menendez, Catalan Institute of Oncology (Spain)
Eleonora Candi, Univ. Roma Tor Vergata (Italy)
Alessandra Baracca Univ. Bologna (Italy)

TREATMENT OF CHRONIC MYELOID LEUKEMIA BY INHIBITION OF TUBULIN POLYMERIZATION

GIUSEPPE LA REGINA
RESEARCH AREA: Novel therapeutic interventions

Dipartimento di Chimica e Tecnologie del Farmaco
Sapienza Università di Roma, Rome, Italy
giuseppe.laregina@uniroma1.it

We designed and synthesized 15 new aroyl diheterocyclic pyrroles (ARDHEPs) as new inhibitors of tubulin assembly by binding the colchicine site. Among the new derivatives, compound (4-(furan-2-yl)-1-(pyrimidin-2-yl)-1*H*-pyrrol-3-yl)(3,4,5-trimethoxyphenyl)-methanone strongly inhibited U-87 MG, OVCAR-3, and MCF-7 cancer cells, induced an increase of cleaved PARP, but was not toxic for normal human primary T lymphocytes at 0.1 µM. Analysis of the levels of lactoperoxidase, malondialdehyde, lactic acid, total glutathione, and ATP suggested that the *in vivo* inhibition of cancer cell proliferation by the same derivative went through stimulation of oxidative stress injury and Fe^{2+} accumulation. Quantitative polymerase chain reaction analysis of the mRNA expression in U-87 MG and SKOV-3 tumour tissues from 4-(furan-2-yl)-1-(pyrimidin-2-yl)-1*H*-pyrrol-3-yl)(3,4,5-trimethoxyphenyl)-methanone-treated mice showed the presence of Ptgs2/Nfe2l2/Sat1/Akr1c1/Gpx4 genes correlated with ferroptosis in both groups. Immunofluorescence staining revealed significantly lower expressions of proteins Ki67, CD31, and ferroptosis negative regulation proteins glutathione peroxidase 4 (GPX4) and FTH1. The same derivative was found to be metabolically stable when incubated with human liver microsomes and showed a medium intrinsic clearance of 36 µL/min/mg protein. In summary, we described the synthesis and antitumor activities *in vitro* and *in vivo* of a new tubulin polymerization inhibitor that induced cell death and presented the typical hallmarks of ferroptosis rather than conventional apoptosis. The biological profile of the new derivative, together with its stability in the presence of human liver microsome enzymes, highlights the new compound as a robust lead compound for further optimization to provide new anticancer drugs based on alternative mechanisms of action.

Furthermore, we designed and synthesized a new additional inhibitor of tubulin polymerization by binding the colchicine site, (1-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrrol-3-yl)(3,4,5-trimethoxy-phenyl)methanone (RS6077). The new derivative inhibited the growth of multiple cancer cell lines, with IC_{50} values in the nM range, without affecting the growth of non-transformed cells. The novel agent arrested cells in the G2/M phase of the cell cycle in both transformed and non-transformed cell lines, but single cell analysis by time-lapse video recording revealed a remarkable selectivity in cell death induction by the new compound: in RPE-1 non-transformed cells mitotic arrest induced was not necessarily followed by cell death; in contrast, in HeLa transformed and in lymphoid-derived transformed AHH1 cell lines, cell death was effectively induced during mitotic arrest in cells that fail to complete mitosis. Importantly, the new agent also

inhibited the growth of the lymphoma TMD8 xenograft model. Together, these findings suggest that the new compound has a selective efficacy in transformed vs non-transformed cells and indicate that the same compound has potential as novel therapeutic agent to treat lymphomas. RS6077 showed good metabolic stability upon incubation with human liver microsomes. In conclusion, our findings with the new pyrrole derivative provides a rationale for further modifications of its core structure to obtain biologically active molecules with still greater anti-cancer activity.

The anti-chronic myeloid leukemia activity of compounds (4-(furan-2-yl)-1-(pyrimidin-2-yl)-1*H*-pyrrol-3-yl)(3,4,5-trimethoxyphenyl)methanone and (1-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrrol-3-yl)(3,4,5-trimethoxy-phenyl)methanone is under biological evaluation. Based on previously reported results, we are confident that both compounds will exhibit an excellent anti-leukemia activity.

Publications

Puxeddu M, Wu J, Bai R, D'Ambrosio M, Nalli M, Coluccia A, Manetto S, Ciogli A, Masci D, Urbani A, Fionda C, Coni S, Bordone R, Canettieri G, Bigogno C, Dondio G, Hamel E, Liu T, Silvestri R, La Regina G. ***Induction of Ferroptosis in Glioblastoma and Ovarian Cancers by a New Pyrrole Tubulin Assembly Inhibitor.*** J Med Chem 2022; **65:15805**–15818, doi: 10.1021/acs.jmedchem.2c01457, IF 8.039.

Sebastiani S, Puxeddu M, Nalli M, Bai R, Altieri L, Rovella P, Gaudio E, Trisciuoglio D, Spriano F, Lavia P, Fionda C, Masci D, Urbani A, Bigogno C, Dondio G, Hamel E, Bertoni F, Silvestri R, La Regina G. ***RS6077 induces mitotic arrest and selectively activates cell death in human cancer cell lines and in a lymphoma tumor in vivo.*** Eur J Med Chem 2023;**246:** e114997, doi: 10.1016/j.ejmech.2022.114997, IF 7.088.

Coluccia A, Bufano M, La Regina G, Puxeddu M, Toto A, Paone A, Bouzidi A, Musto G, Badolati N, Orlando V, Biagioni S, Masci D, Cantatore C, Cirilli R, Cutruzzolà F, Gianni S, Stornaiuolo M, Silvestri R. ***Anticancer Activity of (S)-5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(1-oxo-1-((pyridin-4-ylmethyl)amino)propan-2-yl)-1H-indole-2-carboxamide (RS4690), a New Dishevelled 1 Inhibitor***. Cancers 2022;**14:** e1358, doi: 10.3390/cancers14051358, IF 6.575.

Di Magno L, Coluccia A, Bufano M, Ripa, S, La Regina G, Nalli M, Di Pastena F, Canettieri G, Silvestri R, Frati L. ***Discovery of novel human lactate dehydrogenase inhibitors: Structure-based virtual screening studies and biological assessment.*** Eur J Med Chem 2022;**240:** e114605, doi: 10.1016/j.ejmech.2022.114605, IF 7.088.

Passirani C, Vessieres A, La Regina G, Link W, Silvestri R. ***Modulating undruggable targets to overcome cancer therapy resistance.*** Drug Resist Update 2022;**60:**e100788, doi: 10.1016/j.drup.2021.100788, IF 22.841.

Nalli M, Masci D, Urbani A, La Regina G, Silvestri R. ***Emerging Direct Targeting β-Catenin Agents.*** Molecules 2022;**27:**e7735, doi: 10.3390/molecules27227735, IF 4.927.

Research Group

Giuseppe La Regina, **Antonio Coluccia**: Researchers.
Michela Puxeddu, **Jessica Sebastiani**, **Marianna Bufano**: PhD Students.

Collaborations

Stefano Biagioni, Dipartimento di Biologia e Biotecnologie "Charles Darwin", Sapienza Università di Roma, Rome, Italy.
Addolorata Maria Luce Coluccia, Polo Oncologico Giovanni Paolo II, Lecce, Italy.
Claudia Martini, Dipartimento di Farmacia, Università di Pisa, Pisa, Italy.
Ernest Hamel, National Institutes of Health, Frederick, United States.
Te Liu, Shanghai University, Shanghai, China.

GLIA AND IMMUNE SYSTEM CROSSTALK IN THE CENTRAL NERVOUS SYSTEM

Cristina Limatola
RESEARCH AREA: Neuroscience

Department of *Physiology and Pharmacology Sapienza University of Rome*
cristina.limatola@uniroma1.it

Bidirectional communication among the nervous and the immune system influences many physiological and pathological conditions (1,2). Often, these neuroimmune interactions are mediated by soluble molecules that play distinct roles in the two systems, namely neurotransmitters and cytokines (3,4). Recently, the growing interest in the role of microglia in the progression of many neurodegenerative diseases is developing in an ever-expedited manner. Microglia are the resident immune cells of the central nervous system (CNS) and sustain normal brain functions continuously monitoring cerebral parenchyma to detect neuronal activities and alteration of homeostatic processes (5-7). Particularly, we investigated the contribution of microglia in the regulation of sleep/wake cycle and in the modulation of synaptic activity in the different day phases (8). Using light as a zeitgeber cue, we studied the effects of microglial depletion with the colony stimulating factor-1 receptor antagonist PLX5622 on the sleep/wake cycle and on hippocampal synaptic transmission in male mice. Our data demonstrate that almost complete microglial depletion increases the duration of NREM sleep and reduces the hippocampal excitatory neurotransmission. The fractalkine receptor CX3CR1 plays a relevant role in these effects, because $cx3cr1^{GFP/GFP}$ mice recapitulate what found in PLX5622-treated mice (8). Our findings suggest that microglia participate in the regulation of sleep, adapting their cx3cr1 expression in response to the light/dark phase, and modulating synaptic activity in a phase-dependent manner.

Furthermore, we characterized the effects of pharmacological microglia depletion, achieved by administration of PLX5622, on hippocampal CA3-CA1 synapses of adult wild type mice (9). Following microglial depletion, we observed a reduction of spontaneous and evoked glutamatergic activity associated with a decrease of dendritic spine density. We also observed the appearance of immature synaptic features and higher levels of plasticity. Microglia depleted mice showed a deficit in the acquisition of the Novel Object Recognition task. PLX-induced synaptic changes were absent in $Cx3cr1^{-/-}$ mice, highlighting the role of CX3CL1/CX3CR1 axis in microglia control of synaptic functioning. Altogether, these data demonstrate that microglia contribute to normal synaptic functioning in the adult brain and that their removal induces reversible changes in organization and activity of glutamatergic synapses (9).

Interestingly, also minocycline, an antibiotic with anti-inflammatory properties that modulates microglial phenotype, has been shown to attenuate learning and memory

deficits in animal models (10). We explored whether minocycline recovers the deficits in cognition in a mouse model of depression. C57BL6/J adult male mice were exposed to two weeks of chronic unpredictable mild stress to induce a depressive-like phenotype. Immediately afterward, mice received either vehicle or minocycline for three weeks in standard housing conditions. Cognitive performance in the place learning test was significantly improved by minocycline, as treated mice displayed a higher number of correct responses when learning novel spatial configurations (10). These findings together support the usefulness of minocycline as a potential treatment for cognitive impairment.

Concerning brain pathologies, microglia and immune system have a pivotal role in the sustainment of inflammation in the CNS. In particular, peripheral immune cells were discovered as pivotal players that promptly participate in amyotrophic lateral sclerosis (ALS), speeding up neurodegeneration during progression of the disease (11). We demonstrate that blocking the extravasation of immune cells in the central nervous system using Natalizumab (NAT), an antibody for the α4 integrin, reduces the level of interferon-γ in the spinal cord of ALS mouse models, such as the $hSOD1^{G93A}$ and $TDP43^{A315T}$ mice, modifying microglia and astrocytes phenotype, increasing motor neuron number and prolonging the survival time (11). Our results establish a central role for the immune cells as drivers of inflammation in ALS.

Moreover, in brain tumors, microglia have a pivotal role in supporting the development and growth of the most aggressive brain tumor Glioblastoma (GBM). Once recruited, microglia acquire a pro-tumoral phenotype characterized by a typical morphology: ameboid in the tumor core and with larger soma and thick branches in the tumor periphery. We investigated the role of Ca^{2+}-activated K^{+} channel (KCa3.1) on the phenotypic shift of microglia at the late stage of GBM growth through in vivo two-photon imaging. We demonstrated that microglia respond promptly to KCa3.1 inhibition using a selective inhibitor of the channel (TRAM-34) in a time-dependent manner by boosting ramified projections attributable to a less hypertrophic phenotype in the tumor core (12).

Altogether, our results identify a central role of microglia and immune cells in orchestrating CNS functions in healthy brain, in brain tumor and in neurodegenerative diseases.

References:

1. Pinho-Ribeiro, F. A. et al. Blocking Neuronal Signaling to Immune Cells Treats Streptococcal Invasive Infection. Cell 173, 1083-1097.e22 (2018).
2. Diaz-Salazar, C. et al. Cell-intrinsic adrenergic signaling controls the adaptive NK cell response to viral infection. J Exp Med 217, e20190549 (2020).
3. Wieduwild, E. et al. β2-adrenergic signals downregulate the innate immune response and reduce host resistance to viral infection. J Exp Med 217, e20190554 (2020).

4. Salvador, A. F., de Lima, K. A. & Kipnis, J. Neuromodulation by the immune system: a focus on cytokines. Nat Rev Immunol 21, 526–541 (2021).
5. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience. (1990) 39:151–70. 10.1016/0306-4522(90)90229-W
6. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury *in vivo*. Nat Neurosci. (2005) 8:752–8. 10.1038/nn1472
7. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. Science. (2005) 308:1314–8. 10.1126/science.1110647
8. Corsi G, Picard K, di Castro MA, Garofalo S, Tucci F, Chece G, Del Percio C, Golia MT, Raspa M, Scavizzi F, Decoeur F, Lauro C, Rigamonti M, Iannello F, Ragozzino DA, Russo E, Bernardini G, Nadjar A, Tremblay ME, Babiloni C, Maggi L, Limatola C. Microglia modulate hippocampal synaptic transmission and sleep duration along the light/dark cycle. Glia. 2022 Jan;70(1):89-105. doi: 10.1002/glia.24090.
9. Basilico B, Ferrucci L, Ratano P, Golia MT, Grimaldi A, Rosito M, Ferretti V, Reverte I, Sanchini C, Marrone MC, Giubettini M, De Turris V, Salerno D, Garofalo S, St-Pierre MK, Carrier M, Renzi M, Pagani F, Modi B, Raspa M, Scavizzi F, Gross CT, Marinelli S, Tremblay MÈ, Caprioli D, Maggi L, Limatola C, Di Angelantonio S, Ragozzino D. Microglia control glutamatergic synapses in the adult mouse hippocampus. Glia. 2022 Jan;70(1):173-195. doi: 10.1002/glia.24101.
10. Poggini S, Lopez MB, Albanese NC, Golia MT, Ibáñez FG, Limatola C, Furhmann M, Lalowski M, Tremblay ME, Maggi L, Kaminska B, Branchi I. Minocycline treatment improves cognitive and functional plasticity in a preclinical mouse model of major depressive disorder. Behav Brain Res. 2023 Mar 12;441:114295. doi: 10.1016/j.bbr.2023.114295.
11. Garofalo S, Cocozza G, Bernardini G, Savage J, Raspa M, Aronica E, Tremblay ME, Ransohoff RM, Santoni A, Limatola C. Blocking immune cell infiltration of the central nervous system to tame Neuroinflammation in Amyotrophic lateral sclerosis. Brain Behav Immun. 2022 Oct;105:1-14. doi: 10.1016/j.bbi.2022.06.004.
12. Massenzio F, Cambiaghi M, Marchiotto F, Boriero D, Limatola C, D'Alessandro G, Buffelli M. *In vivo* morphological alterations of TAMs during KCa3.1 inhibition-by using *in vivo* two-photon time-lapse technology. Front Cell Neurosci. 2022 Dec 15;16:1002487. doi: 10.3389/fncel.2022.1002487.

Publications *IF84,7*

Corsi G, Picard K, di Castro MA, Garofalo S, Tucci F, Chece G, Del Percio C, Golia MT, Raspa M, Scavizzi F, Decoeur F, Lauro C, Rigamonti M, Iannello F, Ragozzino DA, Russo E, Bernardini G, Nadjar A, Tremblay ME, Babiloni C, Maggi L, Limatola C. Microglia modulate hippocampal synaptic transmission and sleep duration along the light/dark cycle. Glia. 2022 Jan;70(1):89-105. IF: 8.073

Basilico B, Ferrucci L, Ratano P, Golia MT, Grimaldi A, Rosito M, Ferretti V, Reverte I, Sanchini C, Marrone MC, Giubettini M, De Turris V, Salerno D, Garofalo S, St-Pierre MK, Carrier M, Renzi M, Pagani F, Modi B, Raspa M, Scavizzi F, Gross CT, Marinelli S, Tremblay MÈ, Caprioli D, Maggi L, Limatola C, Di Angelantonio S, Ragozzino D. Microglia control glutamatergic synapses in the adult mouse hippocampus. Glia. 2022 Jan;70(1):173-195. IF: 8.073

Morotti M, Garofalo S, Cocozza G, Antonangeli F, Bianconi V, Mozzetta C, De Stefano ME, Capitani R, Wulff H, Limatola C, Catalano M, Grassi F. Muscle Damage in Dystrophic mdx Mice Is Influenced by the Activity of Ca^{2+}-Activated $K_{Ca}3.1$ Channels. Life (Basel). 2022 Apr 5;12(4):538. IF: 3.253

Marrocco F, Delli Carpini M, Garofalo S, Giampaoli O, De Felice E, Di Castro MA, Maggi L, Scavizzi F, Raspa M, Marini F, Tomassini A, Nicolosi R, Cason C, Trettel F, Miccheli A, Iebba V, D'Alessandro G, Limatola C. Short-chain fatty acids promote the effect of environmental signals on the gut microbiome and metabolome in mice. Commun Biol. 2022 May 31;5(1):517. IF: 6.548

Garofalo S, Cocozza G, Bernardini G, Savage J, Raspa M, Aronica E, Tremblay ME, Ransohoff RM, Santoni A, Limatola C. Blocking immune cell infiltration of the central nervous system to tame Neuroinflammation in Amyotrophic lateral sclerosis. Brain Behav Immun. 2022 Oct;105:1-14. IF: 19.227

Di Castro MA, Garofalo S, De Felice E, Meneghetti N, Di Pietro E, Mormino A, Mazzoni A, Caleo M, Maggi L, Limatola C. Environmental enrichment counteracts the effects of glioma in primary visual cortex. Neurobiol Dis. 2022 Nov;174:105894. IF: 7.046

Catalano M, Serpe C, Limatola C. Microglial Extracellular Vesicles as Modulators of Brain Microenvironment in Glioma. Int J Mol Sci. 2022 Oct 29;23(21):13165. IF: 6.208

D'Alessandro G, Marrocco F, Limatola C. Microglial cells: Sensors for neuronal activity and microbiota-derived molecules. Front Immunol. 2022 Nov 8;13:1011129. IF: 8.786

Serpe C, Michelucci A, Monaco L, Rinaldi A, De Luca M, Familiari P, Relucenti M, Di Pietro E, Di Castro MA, D'Agnano I, Catacuzzeno L, Limatola C, Catalano M. Astrocytes-Derived Small Extracellular Vesicles Hinder Glioma Growth. Biomedicines. 2022 Nov 17;10(11):2952. IF: 4.757

Morotti M, Gaeta A, Limatola C, Catalano M, Di Castro MA, Grassi F. Early Developmental Changes of Muscle Acetylcholine Receptors Are Little Influenced by Dystrophin Absence in *mdx* Mouse. Life (Basel). 2022 Nov 12;12(11):1861. IF: 3.253

Massenzio F, Cambiaghi M, Marchiotto F, Boriero D, Limatola C, D'Alessandro G, Buffelli M. *In vivo* morphological alterations of TAMs during KCa3.1 inhibition-by using *in vivo* two-photon time-lapse technology. Front Cell Neurosci. 2022 Dec 15;16:1002487. IF: 6.147

Poggini S, Lopez MB, Albanese NC, Golia MT, Ibáñez FG, Limatola C, Furhmann M, Lalowski M, Tremblay ME, Maggi L, Kaminska B, Branchi I. Minocycline treatment improves cognitive and functional plasticity in a preclinical mouse model of major depressive disorder. Behav Brain Res. 2023 Mar 12;441:114295. IF: 3.352

Research Group

Myriam Catalano
Giuseppina D’Alessandro
Flavia Trettel
Laura Maggi
Stefano Garofalo
Maria Amalia Di Castro
Germana Cocozza

Collaborations

Angela Santoni
Giovanni Bernardini
Richard M Ransohoff
Marie Eve Tremblay
Fiorenzo Conti
Ling Peng
Eleonora Aronica

NRF2 ACTIVATORS AS INNOVATIVE CELL-TARGETED APPROACHES AGAINST RESPIRATORY VIRUS INFECTIONS

ANNA TERESA PALAMARA
RESEARCH AREA: Infectious agents and associated diseases

Department of Public Health and Infectious Diseases
annateresa.palamara@uniroma1.it

Nuclear factor erythroid 2-related factor 2 (Nrf2) is the transcriptional master regulator of antioxidant responses. In presence of oxidative stress, Nrf2 translocates to the nucleus where it binds to the antioxidant-response element (ARE) and initiates the expression of a variety of cytoprotective genes. In addition to antioxidant responses, Nrf2 is involved in the regulation of many other cellular processes, such as metabolism and inflammation. Most respiratory viruses, including influenza virus and coronavirus, cause oxidative stress in host cells by producing reactive oxygen species (ROS) to maintain a cell microenvironment useful for their replication. Furthermore, several evidence including ours, reported the inhibition of Nrf2 expression upon viral infections to maintain pro-oxidant conditions. We have also recently reported a down-regulation of Nrf2-mediated antioxidant response in respiratory syncytial virus (RSV)-infected, hospitalized children, showing higher disease severity than patients infected with human rhinovirus.
Based on this evidence, the first period of the project was aimed at deepening the mechanism through which respiratory viruses modulate Nrf2 expression and then at evaluating whether Nrf2 activators are able to impair viral replication.
To this aim, we infected permissive cell lines with influenza (A/Puerto Rico/8/34 H1N1) virus or with seasonal (HCoV 229E) or pandemic (SARS-CoV-2) coronaviruses and measured redox parameters including intracellular glutathione (GSH) levels, Nrf2 activation and its antioxidant related genes.
We demonstrated that influenza virus causes a down-modulation of Nrf2-antioxidant response, evaluated as Nrf2 and glucose-6-phosphate dehydrogenase (G6PD) expression and activity, that in turn leads to an increase in oxidative stress and virus replication. Furthermore, in these cells we observed a down-regulation of sirtuin 2 (SIRT2), a NADPH-dependent deacetylase that regulates G6PD activity, an enzyme involved in GSH restoration.
In the second period of the project, we evaluated different compounds known to be able to restore the expression and activation of Nrf2, such as low molecular weight monothiol or dithiol; epigenetic modulators, including inhibitors of histone acetyltransferase (HAT) P300 or histone deacetylase HDAC class I. Each compound was added at different times post-infection.
We found that both thiol agents were able to impair the infectivity of influenza virus when added for 24 hours post-infection. The analysis of redox parameters showed a rescue of the antioxidant response in terms of Nrf2-related antioxidant genes expression as well as GSH

production in infected treated cells. The same compounds were also able to impair SARS-CoV-2 infection by inhibiting the interaction with ACE2 receptor and to decrease viral replication of both pandemic and seasonal coronaviruses when added post-infection for 24 hours.

The use of epigenetic modulators decreased influenza virus titre and viral protein synthesis at different times post-infection indicating a possible role of iHDAC and iHAT in the restoration of gene expression of intracellular factors modulated by the virus and useful for its replication.

Indeed, in infected cells, the use of iHAT P300 restored the acetylation of SIRT2 and rescued the expression and activity of G6PD. Nrf2 mRNA levels and the expression of its target genes were increased in infected cells treated with both HDAC and HAT inhibitors. The use of iHAT and iHDAC were also able to reduce HCoV 229E replication during a time course of infection.

In conclusion, our data indicate that the activation of Nrf2 pathway leads to restoration of intracellular reducing conditions, thus counteracting respiratory virus replication. This approach may open to innovative cell-targeted strategies characterized by broad-spectrum activity against different viruses.

Parallel studies on the potential role of herpes simplex virus-1 (HSV-1) in neurodegenerative processes related to Alzheimer's disease (AD) have proceeded in collaboration with the Institute of Translational Pharmacology of the National Research Council (Dr. De Chiara) and Università Cattolica del Sacro Cuore di Roma (Prof. Grassi team at the Neuroscience Department). Specifically, in vitro and in vivo studies are ongoing to evaluate whether HSV-1 exploits extracellular vesicles to disseminate virus-induced neurodegenerative markers in the brain as well as infection (Protto et al, manuscript in preparation) and the role of proinflammatory citokines in HSV-1-induced AD related markers (Li Puma et al, submitted manuscript).

Along this context, the potential antiviral activity of amphibian antimicrobial peptides (AMPs) belonging to the temporin family was studied. We focused the attention on temporin G (TG), showing that it strongly affects HSV-1 replication by acting either directly on the virion or during the earliest stages of its life cycle, likely through an interaction with HSV-1 glycoprotein B. We also tested the potential antiviral activity of TG against another neurotropic virus, John Cunningham polyomavirus (JCPyV), and found that TG reduced JCPyV infection through direct interaction with the viral capsid protein VP1.

Publications

Sorrentino L., Toscanelli W., Fracella M., De Angelis M., Frasca F., Scagnolari C., Petrarca L., Nenna R., Midulla F., Palamara A.T., Nencioni L., Pierangeli A. **NRF2 antioxidant response and Interferon-stimulated genes are differentially expressed in respiratory syncytial virus and rhinovirus infected hospitalized children.** *Pathogens* 2023 IF: 4.531.

Fraternale A., De Angelis M., De Santis R., Amatore D., Masini S., Monittola F., Menotta M., Biancucci F., Bartoccini F., Retini M., Fiori V., Fioravanti R., Magurano F., Chiarantini L., Lista R.F., Piersanti G., Palamara A.T., Nencioni L., Magnani M., Crinelli R. **Targeting SARS-CoV-2 by synthetic dual-acting thiol compounds that inhibit Spike/ACE2 interaction and viral protein production.** *FASEB J* 2023 37, e22741 IF: 5.834

Checconi P., Coni C., Limongi D., Baldelli S., Ciccarone F., De Angelis M., Mengozzi M., Ghezzi P., Ciriolo M.R., Nencioni L., Palamara A.T. **Influenza virus replication is affected by glutaredoxin1-mediated protein deglutathionylation.** *FASEB J* 2023 37, e22729 IF: 5.834

De Angelis M., Amatore D., Checconi P., Zevini A., Fraternale A., Magnani M., Hiscott J., De Chiara G., Palamara A.T., Nencioni L. **Influenza virus down-modulates G6PD expression and activity to induce oxidative stress and promote its replication.** *Frontiers Cell Infect Microbiol.* 2022 11, Article 804976 IF: 6.073

De Angelis M., De Filippis B., Balaha M., Giampietro L., Miteva M.T., De Chiara G., Palamara A.T., Nencioni L., Mollica A. **Nitrostilbenes: Synthesis and Biological Evaluation as Potential Anti-Influenza Virus Agents.** *Pharmaceuticals* 2022 15, 1061 IF: 5.215

Marcocci M.E., Jackowska B.G., Prezioso C., Protto V., De Angelis M., Di Leva F.S., Casciaro B., Carotenuto A., Mangoni M.L., Palamara A.T., Pietropaolo V., De Chiara G., Nencioni L. **The Inhibition of DNA Viruses by the Amphibian Antimicrobial Peptide Temporin G: A Virological Study Addressing HSV-1 and JCPyV.** *IJMS* 2022 23, 7194 IF: 6.208

Protto V., Marcocci M.E., Miteva M.T., Piacentini R., Li Puma D.D., Grassi C., Palamara A.T., De Chiara G. **Role of HSV-1 in Alzheimer's disease pathogenesis: A challenge for novel preventive/therapeutic strategies.** *Curr Opin Pharmacol.* 2022 63, 10220 IF: 4.768

D'Auria F.D., Casciaro B., De Angelis M., Marcocci M.E., Palamara A.T., Nencioni L., Mangoni M.L. **Antifungal Activity of the Frog Skin Peptide Temporin G and Its Effect on Candida albicans Virulence Factors.** *IJMS* 2022 23, 6345 IF: 6.208

Research Group

Lucia Nencioni Associate Professor
Marta De Angelis Researcher
Maria Elena Marcocci Researcher
Virginia Protto Researcher
Walter Toscanelli PhD student
Mariya Timotey Miteva PhD student

Collaborations

Giovanna De Chiara, Institute of Translational Pharmacology, National Research Council, Rome, Italy
Mauro Magnani, Dept. Biomolecular Sciences, University of Urbino "Carlo Bo", Italy
Maria Luisa Mangoni, Dept. Biochemistry, "Sapienza" University of Rome, Italy
John Hiscott, Pasteur Laboratory, Istituto Pasteur Italia—Fondazione Cenci Bolognetti, Rome, Italy
Florigio Lista, Scientific Dept., Army Medical Center, Rome, Italy
Claudio Grassi, Dept. of Neuroscience, Cattolica University of Rome, Italy
Magurano Fabio, Dept. of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
Adriano Mollica, Dept. of Pharmacy, University of Chieti-Pescara "G. d'Annunzio", Chieti, Italy

INNATE IMMUNITY AS A FIRST LINE OF DEFENCE AGAINST INFECTIONS AND TUMORS

SILVANO SOZZANI
RESEARCH AREA: Immunology

Department of Molecular Medicine
silvano.sozzani@uniroma1.it

Our research activities focussed on the role of innate immune cells in the response against infectious agents and in anticancer immune surveillance. In particular, our research was concentrated on the role of dendritic cells (DC) in the activation of adaptive immunity and of Natural Killer (NK) cells as innate cytotoxic effector cells. Both cell types function at the forefront of immune defences and may reciprocally influence their function. DCs are professional antigen presenting cells displaying the unique capability to activate naïve T cells. However, once activated, DCs are also powerful producers of mediators of inflammation, including pro-inflammatory cytokines. Because of this complex role, any imbalance in DC function reflects into defective or exaggerated immune response and tissue damage. DCs comprise two main subsets, namely conventional or classical DCs (cDCs), that are dedicated antigen presenting cells, and plasmacytoid DCs (pDCs), that mostly respond to viral infections by releasing high amounts of type I interferons. We have focussed our attention on some of the intracellular mechanisms involved in DC activation and on the molecular pathways regulating their localization in peripheral tissues and secondary lymphoid organs. NK cells have long been considered as key components of early host immunity against viruses based on their ability to kill infected cells and produce IFN-g. Cytokines produced by cells of the innate immune compartment, such as type I interferons, IL-18 and IL-12, represent major drivers for the production of IFN-g. In their complex, these cytokines promote a state of antiviral activity, but may also exacerbate a pro-inflammatory phenotype, as observed in Covid-19 patients. Thus, in pathological conditions, the balance between protective immunity and immunopathology might depend on the levels of anti-inflammatory and pro-inflammatory mechanisms. A balanced combination of pro- and anti-inflammatory mediators would facilitate viral clearance and immunity to reinfection, with minimal damage to host tissues.

Publications

Barroeta Seijas AB, Simonetti S, Filippi I, Naldini A, Favaretto G, Colombo T, Natalini A, Antonangeli F, Laffranchi M, Sozzani S, Santoni A, Di Rosa F. ***Mouse dendritic cells in the steady state: Hypoxia, autophagy, and stem cell factor***. Cell Biochem Funct. 2022; 40:718-728. doi: 10.1002/cbf.3737, IF: 3.963

Fionda C, Scarno G, Stabile H, Molfetta R, Di Censo C, Gismondi A, Paolini R, Sozzani S, Santoni A, Sciumè G. ***NK Cells and Other Cytotoxic Innate Lymphocytes in Colorectal Cancer Progression and Metastasis***. Int J Mol Sci. 2022; 23:7859. doi: 10.3390/ijms23147859, IF: 6.208

Monaci S, Coppola F, Rossi D, Giuntini G, Filippi I, Marotta G, Sozzani S, Carraro F, Naldini A. ***Hypoxia Induces Autophagy in Human Dendritic Cells: Involvement of Class III PI3K/Vps34***. Cells. 2022; 11:1695. doi: 10.3390/cells11101695, IF: 7.666

Nguyen HO, Salvi V, Tiberio L, Facchinetti F, Govoni M, Villetti G, Civelli M, Barbazza I, Gaudenzi C, Passari M, Schioppa T, Sozio F, Del Prete A, Sozzani S, Bosisio D. ***The PDE4 inhibitor tanimilast shows distinct immunomodulatory properties associated with a type 2 endotype and CD141 upregulation.*** J Transl Med. 2022; 20:203. doi: 10.1186/s12967-022-03402-x. IF: 8.448

Bufano M, Laffranchi M, Sozzani S, Raimondo D, Silvestri R, Coluccia A. **Exploring CCRL2 chemerin binding using accelerated molecular dynamics.** Proteins. 2022; 90:1714-1720. doi: 10.1002/prot.26348, IF: 4.088

Nguyen HO, Schioppa T, Tiberio L, Facchinetti F, Villetti G, Civelli M, Del Prete A, Sozio F, Gaudenzi C, Passari M, Barbazza I, Sozzani S, Salvi V, Bosisio D. ***The PDE4 Inhibitor Tanimilast Blunts Proinflammatory Dendritic Cell Activation by SARS-CoV-2 ssRNAs.*** Front Immunol. 2022; 12:797390. doi: 10.3389/fimmu.2021.797390, IF: 8.787

Fionda C, Ruggeri S, Sciumè G, Laffranchi M, Quinti I, Milito C, Palange P, Menichini I, Sozzani S, Frati L, Gismondi A, Santoni A, Stabile H. ***Age-dependent NK cell dysfunctions in severe COVID-19 patients***. Front Immunol. 2022; 13:1039120. doi: 10.3389/fimmu.2022.1039120, IF: 8.787

Research Group

Angela Gismondi, Researcher
Giuseppe Sciumè, Researcher
Giovanni Bernardini, Researcher
Helena Stabile, Researcher
Cinzia Fionda, Researcher
Mattia Laffranchi, post-doc
Francesca Sozio, Lab assistant
Arianna Piserà, PhD student

Collaborations

Daniela Bosisio, University of Brescia
Annalisa Del Prete, University of Brescia
Antonella Naldini, University of Siena
Domenico Raimondo, Sapienza University of Rome
Romano Silvestri, Sapienza University of Rome

PUBLICATIONS
(Derived by studies funded by Istituto Pasteur Italia in the year 2022)

1
When killers become thieves: Trogocytosed PD-1 inhibits NK cells in cancer.
Hasim MS, Marotel M, Hodgins JJ, Vulpis E, Makinson OJ, Asif S, Shih HY, Scheer AK, MacMillan O, Alonso FG, Burke KP, Cook DP, Li R, Petrucci MT, Santoni A, Fallon PG, Sharpe AH, Sciumè G, Veillette A, Zingoni A, Gray DA, McCurdy A, Ardolino M. Sci Adv. 2022 Apr 15;8(15):eabj3286. doi: 10.1126/sciadv.abj3286. Epub 2022 Apr 13.
PMID: 35417234
2
Microglia control glutamatergic synapses in the adult mouse hippocampus.
Basilico B, Ferrucci L, Ratano P, Golia MT, Grimaldi A, Rosito M, Ferretti V, Reverte I, Sanchini C, Marrone MC, Giubettini M, De Turris V, Salerno D, Garofalo S, St-Pierre MK, Carrier M, Renzi M, Pagani F, Modi B, Raspa M, Scavizzi F, Gross CT, Marinelli S, Tremblay MÈ, Caprioli D, Maggi L, Limatola C, Di Angelantonio S, Ragozzino D. Glia. 2022 Jan;70(1):173-195. doi: 10.1002/glia.24101. Epub 2021 Oct 18. PMID: 34661306
3
Neuro-Signals from Gut Microbiota: Perspectives for Brain Glioma.
D'Alessandro G, Lauro C, Quaglio D, Ghirga F, Botta B, Trettel F, Limatola C. Cancers (Basel). 2021 Jun 4;13(11):2810. doi: 10.3390/cancers13112810. PMID: 34199968
4
m6A RNA methylation and beyond - The epigenetic machinery and potential treatment options.
Garbo S, Zwergel C, Battistelli C. Drug Discov Today. 2021 Nov;26(11):2559-2574. doi: 10.1016/j.drudis.2021.06.004. Epub 2021 Jun 12. PMID: 34126238
5
CD8+ T Cell Senescence: Lights and Shadows in Viral Infections, Autoimmune Disorders and Cancer.
Tedeschi V, Paldino G, Kunkl M, Paroli M, Sorrentino R, Tuosto L, Fiorillo MT. Int J Mol Sci. 2022 Mar 21;23(6):3374. doi: 10.3390/ijms23063374. PMID: 35328795
6
Microglia modulate hippocampal synaptic transmission and sleep duration along the light/dark cycle.
Corsi G, Picard K, di Castro MA, Garofalo S, Tucci F, Chece G, Del Percio C, Golia MT, Raspa M, Scavizzi F, Decoeur F, Lauro C, Rigamonti M, Iannello F, Ragozzino DA, Russo E, Bernardini G, Nadjar A, Tremblay ME, Babiloni C, Maggi L, Limatola C. Glia. 2022 Jan;70(1):89-105. doi: 10.1002/glia.24090. Epub 2021 Sep 6. PMID: 34487590

7
Emerging Direct Targeting β-Catenin Agents.
Nalli M, Masci D, Urbani A, La Regina G, Silvestri R. Molecules. 2022 Nov 10;27(22):7735. doi: 10.3390/molecules27227735. PMID: 36431838
8
Blocking immune cell infiltration of the central nervous system to tame Neuroinflammation in Amyotrophic lateral sclerosis.
Garofalo S, Cocozza G, Bernardini G, Savage J, Raspa M, Aronica E, Tremblay ME, Ransohoff RM, Santoni A, Limatola C. Brain Behav Immun. 2022 Oct;105:1-14. doi: 10.1016/j.bbi.2022.06.004. Epub 2022 Jun 7. PMID: 35688338
9
Repurposing of Trimetazidine for amyotrophic lateral sclerosis: A study in SOD1G93A mice.
Scaricamazza S, Salvatori I, Amadio S, Nesci V, Torcinaro A, Giacovazzo G, Primiano A, Gloriani M, Candelise N, Pieroni L, Loeffler JP, Renè F, Quessada C, Tefera TW, Wang H, Steyn FJ, Ngo ST, Dobrowolny G, Lepore E, Urbani A, Musarò A, Volonté C, Ferraro E, Coccurello R, Valle C, Ferri A. Br J Pharmacol. 2022 Apr;179(8):1732-1752. doi: 10.1111/bph.15738. Epub 2022 Jan 13. PMID: 34783031
10
Editorial: Insights in T Cell Biology: 2021.
Tuosto L. Front Immunol. 2022 Sep 21;13:1039602. doi: 10.3389/fimmu.2022.1039602. eCollection 2022. PMID: 36238305
11
The hormetic and hermetic role of IL-6.
Forcina L, Franceschi C, Musarò A. Ageing Res Rev. 2022 Sep;80:101697. doi: 10.1016/j.arr.2022.101697. Epub 2022 Jul 16. PMID: 35850167
12
Impact on NK cell functions of acute versus chronic exposure to extracellular vesicle-associated MICA: Dual role in cancer immunosurveillance.
Vulpis E, Loconte L, Peri A, Molfetta R, Caracciolo G, Masuelli L, Tomaipitinca L, Peruzzi G, Petillo S, Petrucci MT, Fazio F, Simonelli L, Fionda C, Soriani A, Cerboni C, Cippitelli M, Paolini R, Bernardini G, Palmieri G, Santoni A, Zingoni A. J Extracell Vesicles. 2022 Jan;11(1):e12176. doi: 10.1002/jev2.12176. PMID: 34973063
13
Editorial: The Effects of Enriched Environment on NK cells and Macrophages and Their Underlying Mechanisms.
Kobayashi Y, Limatola C, Gan Y. Front Immunol. 2022 Feb 24;13:862488. doi: 10.3389/fimmu.2022.862488. eCollection 2022. PMID: 35280990
14
T Cell Memory in Infection, Cancer, and Autoimmunity.
Barnaba V. Front Immunol. 2022 Jan 3;12:811968. doi: 10.3389/fimmu.2021.811968. eCollection 2021. PMID: 35069600

15
Editorial: The Genetic and Epigenetic Bases of Cellular Response to Ionizing Radiation.
Seong KM, Cenci G. Front Genet. 2022 Mar 4;13:857168. doi: 10.3389/fgene.2022.857168. eCollection 2022. PMID: 35309150
16
Transposable Elements: Major Players in Shaping Genomic and Evolutionary Patterns.
Colonna Romano N, Fanti L. Cells. 2022 Mar 19;11(6):1048. doi: 10.3390/cells11061048. PMID: 35326499
17
The Revaluation of Plant-Derived Terpenes to Fight Antibiotic-Resistant Infections.
Cappiello F, Loffredo MR, Del Plato C, Cammarone S, Casciaro B, Quaglio D, Mangoni ML, Botta B, Ghirga F. Antibiotics (Basel). 2020 Jun 13;9(6):325. doi: 10.3390/antibiotics9060325. PMID: 32545761
18
Self or Non-Self? It Is also a Matter of RNA Recognition and Editing by ADAR1.
Tassinari V, Cerboni C, Soriani A. Biology (Basel). 2022 Apr 8;11(4):568. doi: 10.3390/biology11040568. PMID: 35453767
19
Astrocytes and Inflammatory T Helper Cells: A Dangerous Liaison in Multiple Sclerosis.
Kunkl M, Amormino C, Tedeschi V, Fiorillo MT, Tuosto L. Front Immunol. 2022 Feb 8;13:824411. doi: 10.3389/fimmu.2022.824411. eCollection 2022. PMID: 35211120
20
Microglial Extracellular Vesicles as Modulators of Brain Microenvironment in Glioma.
Catalano M, Serpe C, Limatola C. Int J Mol Sci. 2022 Oct 29;23(21):13165. doi: 10.3390/ijms232113165. PMID: 36361947
21
Role of HSV-1 in Alzheimer's disease pathogenesis: A challenge for novel preventive/therapeutic strategies.
Protto V, Marcocci ME, Miteva MT, Piacentini R, Li Puma DD, Grassi C, Palamara AT, De Chiara G. Curr Opin Pharmacol. 2022 Apr;63:102200. doi: 10.1016/j.coph.2022.102200. Epub 2022 Mar 8. PMID: 35276497
22
The Role of Skeletal Muscle in Neuromuscular Diseases: From Cellular and Molecular Players to Therapeutic Interventions.
Dobrowolny G, Scicchitano BM. Cells. 2022 Apr 3;11(7):1207. doi: 10.3390/cells11071207. PMID: 35406771

23
The innovative potential of selenium-containing agents for fighting cancer and viral infections.
Ali W, Benedetti R, Handzlik J, Zwergel C, Battistelli C. Drug Discov Today. 2021 Jan;26(1):256-263. doi: 10.1016/j.drudis.2020.10.014. Epub 2020 Oct 23. PMID: 33164821
24
Astrocytes-Derived Small Extracellular Vesicles Hinder Glioma Growth.
Serpe C, Michelucci A, Monaco L, Rinaldi A, De Luca M, Familiari P, Relucenti M, Di Pietro E, Di Castro MA, D'Agnano I, Catacuzzeno L, Limatola C, Catalano M. Biomedicines. 2022 Nov 17;10(11):2952. doi: 10.3390/biomedicines10112952. PMID: 36428520
25
Nutrient Sensing and Biofilm Modulation: The Example of L-arginine in Pseudomonas.
Scribani Rossi C, Barrientos-Moreno L, Paone A, Cutruzzolà F, Paiardini A, Espinosa-Urgel M, Rinaldo S. Int J Mol Sci. 2022 Apr 15;23(8):4386. doi: 10.3390/ijms23084386. PMID: 35457206
26
Identification of the Telomere elongation Mutation in Drosophila.
Reddy HM, Randall TA, Cipressa F, Porrazzo A, Cenci G, Frydrychova RC, Mason JM. Cells. 2022 Nov 3;11(21):3484. doi: 10.3390/cells11213484. PMID: 36359878
27
Chronic Cancer Pain: Opioids within Tumor Microenvironment Affect Neuroinflammation, Tumor and Pain Evolution.
Santoni A, Santoni M, Arcuri E. Cancers (Basel). 2022 Apr 30;14(9):2253. doi: 10.3390/cancers14092253. PMID: 35565382
28
What microglia depletion approaches tell us about the role of microglia on synaptic function and behavior.
Basilico B, Ferrucci L, Khan A, Di Angelantonio S, Ragozzino D, Reverte I. Front Cell Neurosci. 2022 Nov 4;16:1022431. doi: 10.3389/fncel.2022.1022431. eCollection 2022. PMID: 36406752
29
The role of lipid metabolism in shaping the expansion and the function of regulatory T cells.
Pinzon Grimaldos A, Bini S, Pacella I, Rossi A, Di Costanzo A, Minicocci I, D'Erasmo L, Arca M, Piconese S. Clin Exp Immunol. 2022 Jun 11;208(2):181-192. doi: 10.1093/cei/uxab033. PMID: 35020862
30
Naturally-Occurring Alkaloids of Plant Origin as Potential Antimicrobials against Antibiotic-Resistant Infections.
Casciaro B, Mangiardi L, Cappiello F, Romeo I, Loffredo MR, Iazzetti A, Calcaterra A, Goggiamani A, Ghirga F, Mangoni ML, Botta B, Quaglio D. Molecules. 2020 Aug 9;25(16):3619. doi: 10.3390/molecules25163619. PMID: 32784887

31
An Overview of the Molecular Mechanisms Associated with Myocardial Ischemic Injury: State of the Art and Translational Perspectives.
Schirone L, Forte M, D'Ambrosio L, Valenti V, Vecchio D, Schiavon S, Spinosa G, Sarto G, Petrozza V, Frati G, Sciarretta S. Cells. 2022 Mar 30;11(7):1165. doi: 10.3390/cells11071165. PMID: 35406729
32
Exploring CCRL2 chemerin binding using accelerated molecular dynamics.
Bufano M, Laffranchi M, Sozzani S, Raimondo D, Silvestri R, Coluccia A. Proteins. 2022 Sep;90(9):1714-1720. doi: 10.1002/prot.26348. Epub 2022 Apr 29. PMID: 35437825
33
CAPE and its synthetic derivative VP961 restore BACH1/NRF2 axis in Down Syndrome.
Pagnotta S, Tramutola A, Barone E, Di Domenico F, Pittalà V, Salerno L, Folgiero V, Caforio M, Locatelli F, Petrini S, Butterfield DA, Perluigi M. Free Radic Biol Med. 2022 Apr;183:1-13. doi: 10.1016/j.freeradbiomed.2022.03.006. Epub 2022 Mar 11. PMID: 35283228
34
NK Cells and Other Cytotoxic Innate Lymphocytes in Colorectal Cancer Progression and Metastasis.
Fionda C, Scarno G, Stabile H, Molfetta R, Di Censo C, Gismondi A, Paolini R, Sozzani S, Santoni A, Sciumè G. Int J Mol Sci. 2022 Jul 16;23(14):7859. doi: 10.3390/ijms23147859. PMID: 35887206
35
Stromal and Immune Cell Dynamics in Tumor Associated Tertiary Lymphoid Structures and Anti-Tumor Immune Responses.
Rossi A, Belmonte B, Carnevale S, Liotti A, De Rosa V, Jaillon S, Piconese S, Tripodo C. Front Cell Dev Biol. 2022 Jul 8;10:933113. doi: 10.3389/fcell.2022.933113. eCollection 2022. PMID: 35874810
36
SARS-CoV-2 Spike Does Not Possess Intrinsic Superantigen-like Inflammatory Activity.
Amormino C, Tedeschi V, Paldino G, Arcieri S, Fiorillo MT, Paiardini A, Tuosto L, Kunkl M. Cells. 2022 Aug 15;11(16):2526. doi: 10.3390/cells11162526. PMID: 36010602
37
Arabidopsis thaliana serine hydroxymethyltransferases: functions, structures, and perspectives.
Nogués I, Sekula B, Angelaccio S, Grzechowiak M, Tramonti A, Contestabile R, Ruszkowski M. Plant Physiol Biochem. 2022 Jul 31;187:37-49. doi: 10.1016/j.plaphy.2022.07.025. Online ahead of print. PMID: 35947902

38
MALAT1-dependent hsa_circ_0076611 regulates translation rate in triple-negative breast cancer.
Turco C, Esposito G, Iaiza A, Goeman F, Benedetti A, Gallo E, Daralioti T, Perracchio L, Sacconi A, Pasanisi P, Muti P, Pulito C, Strano S, Ianniello Z, Fatica A, Forcato M, Fazi F, Blandino G, Fontemaggi G. Commun Biol. 2022 Jun 16;5(1):598. doi: 10.1038/s42003-022-03539-x. PMID: 35710947
39
The Coronavirus pandemic - 2022: Viruses, variants & vaccines.
Alexandridi M, Mazej J, Palermo E, Hiscott J. Cytokine Growth Factor Rev. 2022 Feb;63:1-9. doi: 10.1016/j.cytogfr.2022.02.002. Epub 2022 Feb 12. PMID: 35216872
40
Heterocycle-containing tranylcypromine derivatives endowed with high anti-LSD1 activity.
Fioravanti R, Rodriguez V, Caroli J, Chianese U, Benedetti R, Di Bello E, Noce B, Zwergel C, Corinti D, Viña D, Altucci L, Mattevi A, Valente S, Mai A. J Enzyme Inhib Med Chem. 2022 Dec;37(1):973-985. doi: 10.1080/14756366.2022.2052869. PMID: 35317680
41
Spatially resolved transcriptomics reveals innervation-responsive functional clusters in skeletal muscle.
D'Ercole C, D'Angelo P, Ruggieri V, Proietti D, Virtanen L, Parisi C, Riera CS, Renzini A, Macone A, Marzullo M, Ciapponi L, Bonvissuto D, Sette C, Giordani L, Madaro L. Cell Rep. 2022 Dec 20;41(12):111861. doi: 10.1016/j.celrep.2022.111861. PMID: 36543136
42
Nitrostilbenes: Synthesis and Biological Evaluation as Potential Anti-Influenza Virus Agents.
De Angelis M, De Filippis B, Balaha M, Giampietro L, Miteva MT, De Chiara G, Palamara AT, Nencioni L, Mollica A. Pharmaceuticals (Basel). 2022 Aug 26;15(9):1061. doi: 10.3390/ph15091061. PMID: 36145282
43
Cytological heterogeneity of heterochromatin among 10 sequenced Drosophila species.
Marchetti M, Piacentini L, Berloco MF, Casale AM, Cappucci U, Pimpinelli S, Fanti L. Genetics. 2022 Sep 30;222(2):iyac119. doi: 10.1093/genetics/iyac119. PMID: 35946576
44
SYNCRIP Modulates the Epithelial-Mesenchymal Transition in Hepatocytes and HCC Cells.
Riccioni V, Trionfetti F, Montaldo C, Garbo S, Marocco F, Battistelli C, Marchetti A, Strippoli R, Amicone L, Cicchini C, Tripodi M. Int J Mol Sci. 2022 Jan 14;23(2):913. doi: 10.3390/ijms23020913. PMID: 35055098

45
Editorial: Secondary Metabolites and Peptides as Unique Natural Reservoirs of New Therapeutic Leads for Treatment of Cancer and Microbial Infections.
Mangoni ML, Bhunia A, Botta B, Ghirga F. Front Chem. 2021 Aug 12;9:748180. doi: 10.3389/fchem.2021.748180. eCollection 2021. PMID: 34458241
46
An overview on tumor treating fields (TTFields) technology as a new potential subsidiary biophysical treatment for COVID-19.
Farmani AR, Mahdavinezhad F, Scagnolari C, Kouhestani M, Mohammadi S, Ai J, Shoormeij MH, Rezaei N. Drug Deliv Transl Res. 2022 Jul;12(7):1605-1615. doi: 10.1007/s13346-021-01067-5. Epub 2021 Sep 20. PMID: 34542840
47
Environmental enrichment counteracts the effects of glioma in primary visual cortex.
Di Castro MA, Garofalo S, De Felice E, Meneghetti N, Di Pietro E, Mormino A, Mazzoni A, Caleo M, Maggi L, Limatola C. Neurobiol Dis. 2022 Nov;174:105894. doi: 10.1016/j.nbd.2022.105894. Epub 2022 Oct 12. PMID: 36240950
48
Trogocytosis in innate immunity to cancer is an intimate relationship with unexpected outcomes.
Mattei F, Andreone S, Spadaro F, Noto F, Tinari A, Falchi M, Piconese S, Afferni C, Schiavoni G. iScience. 2022 Sep 12;25(10):105110. doi: 10.1016/j.isci.2022.105110. eCollection 2022 Oct 21. PMID: 36185368
49
Hypoxia Induces Autophagy in Human Dendritic Cells: Involvement of Class III PI3K/Vps34.
Monaci S, Coppola F, Rossi D, Giuntini G, Filippi I, Marotta G, Sozzani S, Carraro F, Naldini A. Cells. 2022 May 19;11(10):1695. doi: 10.3390/cells11101695. PMID: 35626732
50
Egg-adaptive mutations of human influenza H3N2 virus are contingent on natural evolution.
Liang W, Tan TJC, Wang Y, Lv H, Sun Y, Bruzzone R, Mok CKP, Wu NC. PLoS Pathog. 2022 Sep 26;18(9):e1010875. doi: 10.1371/journal.ppat.1010875. eCollection 2022 Sep. PMID: 36155668
51
Cytosolic localization and in vitro assembly of human de novo thymidylate synthesis complex.
Spizzichino S, Boi D, Boumis G, Lucchi R, Liberati FR, Capelli D, Montanari R, Pochetti G, Piacentini R, Parisi G, Paone A, Rinaldo S, Contestabile R, Tramonti A, Paiardini A, Giardina G, Cutruzzolà F. FEBS J. 2022 Mar;289(6):1625-1649. doi: 10.1111/febs.16248. Epub 2021 Nov 12. PMID: 34694685

52
Analysis of viral nucleic acids in duodenal biopsies from adult patients with celiac disease.
Calabretto M, Di Carlo D, Falasca F, Mazzuti L, Meacci A, Donato G, Greco N, Mezzatesta L, Morrone A, Turriziani O, Picarelli A. Eur J Gastroenterol Hepatol. 2022 Nov 1;34(11):1107-1110. doi: 10.1097/MEG.0000000000002404. Epub 2022 Jul 14. PMID: 35830367
53
Evolutionary conserved relocation of chromatin remodeling complexes to the mitotic apparatus.
Messina G, Prozzillo Y, Monache FD, Santopietro MV, Dimitri P. BMC Biol. 2022 Aug 3;20(1):172. doi: 10.1186/s12915-022-01365-5. PMID: 35922843
54
Culture of Human iPSC-Derived Motoneurons in Compartmentalized Microfluidic Devices and Quantitative Assays for Studying Axonal Phenotypes.
Garone MG, D'Antoni C, Rosa A. Methods Mol Biol. 2022;2429:189-199. doi: 10.1007/978-1-0716-1979-7_12. PMID: 35507162
55
Ultra-Short Cyclized β-Boomerang Peptides: Structures, Interactions with Lipopolysaccharide, Antibiotic Potentiator and Wound Healing.
Sinha S, Dhanabal VB, Manivannen VL, Cappiello F, Tan SM, Bhattacharjya S. Int J Mol Sci. 2022 Dec 23;24(1):263. doi: 10.3390/ijms24010263. PMID: 36613707
56
Ultrastructural Damages to H1N1 Influenza Virus Caused by Vapor Essential Oils.
Madia VN, Toscanelli W, De Vita D, De Angelis M, Messore A, Ialongo D, Scipione L, Tudino V, D'Auria FD, Di Santo R, Garzoli S, Stringaro A, Colone M, Marchetti M, Superti F, Nencioni L, Costi R. Molecules. 2022 Jun 9;27(12):3718. doi: 10.3390/molecules27123718. PMID: 35744845
57
The PDE4 Inhibitor Tanimilast Blunts Proinflammatory Dendritic Cell Activation by SARS-CoV-2 ssRNAs.
Nguyen HO, Schioppa T, Tiberio L, Facchinetti F, Villetti G, Civelli M, Del Prete A, Sozio F, Gaudenzi C, Passari M, Barbazza I, Sozzani S, Salvi V, Bosisio D. Front Immunol. 2022 Jan 24;12:797390. doi: 10.3389/fimmu.2021.797390. eCollection 2021. PMID: 35140709
58
Allergic rhinitis, microbiota and passive smoke in children: A pilot study.
Brindisi G, Marazzato M, Brunetti F, De Castro G, Loffredo L, Carnevale R, Cinicola B, Palamara AT, Conte MP, Zicari AM. Pediatr Allergy Immunol. 2022 Jan;33 Suppl 27(Suppl 27):22-26. doi: 10.1111/pai.13621. PMID: 35080308

59
Zinc Supplementation Enhances the Pro-Death Function of UPR in Lymphoma Cells Exposed to Radiation.
Gonnella R, Guttieri L, Gilardini Montani MS, Santarelli R, Bassetti E, D'Orazi G, Cirone M. Biology (Basel). 2022 Jan 13;11(1):132. doi: 10.3390/biology11010132. PMID: 35053130
60
The VirF21:VirF30 protein ratio is affected by temperature and impacts Shigella flexneri host cell invasion.
Skovajsová E, Colonna B, Prosseda G, Sellin ME, Di Martino ML. FEMS Microbiol Lett. 2022 Jun 22;369(1):fnac043. doi: 10.1093/femsle/fnac043. PMID: 35521699
61
NKG2D engagement on human NK cells leads to DNAM-1 hypo-responsiveness through different converging mechanisms.
Milito ND, Zingoni A, Stabile H, Soriani A, Capuano C, Cippitelli M, Gismondi A, Santoni A, Paolini R, Molfetta R. Eur J Immunol. 2022 Nov 28. doi: 10.1002/eji.202250198. Online ahead of print. PMID: 36440686
62
Multicomponent Synthesis of Diaminopurine and Guanine PNA's Analogues Active against Influenza A Virus from Prebiotic Compounds.
Bizzarri BM, Fanelli A, Ciprini S, Giorgi A, De Angelis M, Fioravanti R, Nencioni L, Saladino R. ACS Omega. 2022 Nov 29;7(49):45253-45264. doi: 10.1021/acsomega.2c05754. eCollection 2022 Dec 13. PMID: 36530301
63
Thiamine-dependent regulation of mammalian brain pyridoxal kinase in vitro and in vivo.
Bunik V, Aleshin V, Nogues I, Kähne T, Parroni A, Contestabile R, Salvo ML, Graf A, Tramonti A. J Neurochem. 2022 Apr;161(1):20-39. doi: 10.1111/jnc.15576. Epub 2022 Feb 23. PMID: 35050500
64
Muscle Damage in Dystrophic mdx Mice Is Influenced by the Activity of Ca2+-Activated KCa3.1 Channels.
Morotti M, Garofalo S, Cocozza G, Antonangeli F, Bianconi V, Mozzetta C, De Stefano ME, Capitani R, Wulff H, Limatola C, Catalano M, Grassi F. Life (Basel). 2022 Apr 5;12(4):538. doi: 10.3390/life12040538. PMID: 35455028
65
p62/SQSTM1 promotes mitophagy and activates the NRF2-mediated antioxidant and anti-inflammatory response restraining EBV-driven B lymphocyte proliferation.
Gilardini Montani MS, Tarquini G, Santarelli R, Gonnella R, Romeo MA, Benedetti R, Arena A, Faggioni A, Cirone M. Carcinogenesis. 2022 Apr 25;43(3):277-287
doi: 10.1093/carcin/bgab116. PMID: 34958370

66
ANGPTL3 deficiency associates with the expansion of regulatory T cells with reduced lipid content.
Pinzon Grimaldos A, Pacella I, Bini S, Tucci G, Cammarata I, Di Costanzo A, Minicocci I, D'Erasmo L, Arca M, Piconese S. Atherosclerosis. 2022 Dec;362:38-46. doi: 10.1016/j.atherosclerosis.2022.09.014. Epub 2022 Oct 7. PMID: 36253169
67
Synthetic Amphipathic β-Sheet Temporin-Derived Peptide with Dual Antibacterial and Anti-Inflammatory Activities.
Bellavita R, Buommino E, Casciaro B, Merlino F, Cappiello F, Marigliano N, Saviano A, Maione F, Santangelo R, Mangoni ML, Galdiero S, Grieco P, Falanga A. Antibiotics (Basel). 2022 Sep 21;11(10):1285. doi: 10.3390/antibiotics11101285. PMID: 36289944
68
High Levels of Admixture in Anopheles gambiae Populations from Côte d'Ivoire Revealed by Multilocus Genotyping.
Tondossama N, Coulibaly ZI, Traoré I, Ako BA, Zoh DD, Virgillito C, Guindo-Coulibaly N, Serini P, Assouho FK, Dia I, Touré AO, Adja MA, Caputo B, Della Torre A, Pichler V. Insects. 2022 Nov 26;13(12):1090. doi: 10.3390/insects13121090. PMID: 36555000
69
Baricitinib therapy response in rheumatoid arthritis patients associates to STAT1 phosphorylation in monocytes.
Tucci G, Garufi C, Pacella I, Zagaglioni M, Pinzon Grimaldos A, Ceccarelli F, Conti F, Spinelli FR, Piconese S. Front Immunol. 2022 Jul 25;13:932240. doi: 10.3389/fimmu.2022.932240. eCollection 2022. PMID: 35958600
70
Mouse dendritic cells in the steady state: Hypoxia, autophagy, and stem cell factor.
Barroeta Seijas AB, Simonetti S, Filippi I, Naldini A, Favaretto G, Colombo T, Natalini A, Antonangeli F, Laffranchi M, Sozzani S, Santoni A, Di Rosa F. Cell Biochem Funct. 2022 Oct;40(7):718-728. doi: 10.1002/cbf.3737. Epub 2022 Sep 7. PMID: 36069062
71
Broad-Spectrum Antiviral Activity of the Amphibian Antimicrobial Peptide Temporin L and Its Analogs.
Zannella C, Chianese A, Palomba L, Marcocci ME, Bellavita R, Merlino F, Grieco P, Folliero V, De Filippis A, Mangoni M, Nencioni L, Franci G, Galdiero M. Int J Mol Sci. 2022 Feb 13;23(4):2060. doi: 10.3390/ijms23042060. PMID: 35216177
72
Short-chain fatty acids promote the effect of environmental signals on the gut microbiome and metabolome in mice.
Marrocco F, Delli Carpini M, Garofalo S, Giampaoli O, De Felice E, Di Castro MA, Maggi L, Scavizzi F, Raspa M, Marini F, Tomassini A, Nicolosi R, Cason C, Trettel F, Miccheli A,

Iebba V, D'Alessandro G, Limatola C. Commun Biol. 2022 May 31;5(1):517. doi: 10.1038/s42003-022-03468-9. PMID: 35641653.
73
ATF6 prevents DNA damage and cell death in colon cancer cells undergoing ER stress.
Benedetti R, Romeo MA, Arena A, Gilardini Montani MS, Di Renzo L, D'Orazi G, Cirone M. Cell Death Discov. 2022 Jun 25;8(1):295. doi: 10.1038/s41420-022-01085-3. PMID: 35752616
74
Inhibition of Glycolysis Impairs Retinoic Acid-Inducible Gene I-Mediated Antiviral Responses in Primary Human Dendritic Cells.
Zevini A, Palermo E, Di Carlo D, Alexandridi M, Rinaldo S, Paone A, Cutruzzola F, Etna MP, Coccia EM, Olagnier D, Hiscott J. Front Cell Infect Microbiol. 2022 Jul 18;12:910864. doi: 10.3389/fcimb.2022.910864. eCollection 2022. PMID: 35923800
75
Influenza Virus Down-Modulates G6PD Expression and Activity to Induce Oxidative Stress and Promote Its Replication.
De Angelis M, Amatore D, Checconi P, Zevini A, Fraternale A, Magnani M, Hiscott J, De Chiara G, Palamara AT, Nencioni L. Front Cell Infect Microbiol. 2022 Jan 6;11:804976. doi: 10.3389/fcimb.2021.804976. eCollection 2021. PMID: 35071051
76
Early Developmental Changes of Muscle Acetylcholine Receptors Are Little Influenced by Dystrophin Absence in mdx Mouse.
Morotti M, Gaeta A, Limatola C, Catalano M, Di Castro MA, Grassi F. Life (Basel). 2022 Nov 12;12(11):1861. doi: 10.3390/life12111861. PMID: 36430996
77
Derivatives of Esculentin-1 Peptides as Promising Candidates for Fighting Infections from Escherichia coli O157:H7.
Scotti R, Casciaro B, Stringaro A, Morgia F, Mangoni ML, Gabbianelli R. Antibiotics (Basel). 2022 May 13;11(5):656. doi: 10.3390/antibiotics11050656. PMID: 35625300
78
Genetic Diversity of Antimicrobial Resistance and Key Virulence Features in Two Extensively Drug-Resistant Acinetobacter baumannii Isolates.
Marazzato M, Scribano D, Sarshar M, Brunetti F, Fillo S, Fortunato A, Lista F, Palamara AT, Zagaglia C, Ambrosi C. Int J Environ Res Public Health. 2022 Mar 1;19(5):2870. doi: 10.3390/ijerph19052870. PMID: 35270562

79
Bronchiolitis phenotypes identified by latent class analysis may influence the occurrence of respiratory sequelae.
Petrarca L, Nenna R, Di Mattia G, Frassanito A, Castro-Rodriguez JA, Rodriguez Martinez CE, Mancino E, Arima S, Scagnolari C, Pierangeli A, Midulla F. Pediatr Pulmonol. 2022 Mar;57(3):616-622. doi: 10.1002/ppul.25799. Epub 2022 Jan 11. PMID: 34931488
80
ALS-related FUS mutations alter axon growth in motoneurons and affect HuD/ELAVL4 and FMRP activity.
Garone MG, Birsa N, Rosito M, Salaris F, Mochi M, de Turris V, Nair RR, Cunningham TJ, Fisher EMC, Morlando M, Fratta P, Rosa A. Commun Biol. 2021 Sep 1;4(1):1025. doi: 10.1038/s42003-021-02538-8. PMID: 34471224
81
Structural Elucidation and Antimicrobial Characterization of Novel Diterpenoids from Fabiana densa var. ramulosa.
Quaglio D, Corradi S, Erazo S, Vergine V, Berardozzi S, Sciubba F, Cappiello F, Crestoni ME, Ascenzioni F, Imperi F, Delle Monache F, Mori M, Loffredo MR, Ghirga F, Casciaro B, Botta B, Mangoni ML. ACS Med Chem Lett. 2020 Jan 30;11(5):760-765. doi: 10.1021/acsmedchemlett.9b00605. eCollection 2020 May 14. PMID: 32435382
82
New Inhibitors of the Human p300/CBP Acetyltransferase Are Selectively Active against the Arabidopsis HAC Proteins.
Longo C, Lepri A, Paciolla A, Messore A, De Vita D, Bonaccorsi di Patti MC, Amadei M, Madia VN, Ialongo D, Di Santo R, Costi R, Vittorioso P. Int J Mol Sci. 2022 Sep 9;23(18):10446. doi: 10.3390/ijms231810446. PMID: 36142359
83
An Italian Multicenter Study on the Epidemiology of Respiratory Syncytial Virus During SARS-CoV-2 Pandemic in Hospitalized Children.
Nenna R, Matera L, Licari A, Manti S, Di Bella G, Pierangeli A, Palamara AT, Nosetti L, Leonardi S, Marseglia GL, Midulla F; ICHRIS Group. Front Pediatr. 2022 Jul 14;10:930281. doi: 10.3389/fped.2022.930281. eCollection 2022. PMID: 35911833
84
Dual SMO/BRAF Inhibition by Flavonolignans from Silybum marianum †.
Diukendjieva A, Zaharieva MM, Mori M, Alov P, Tsakovska I, Pencheva T, Najdenski H, Křen V, Felici C, Bufalieri F, Di Marcotullio L, Botta B, Botta M, Pajeva I. Antioxidants (Basel). 2020 May 5;9(5):384. doi: 10.3390/antiox9050384. PMID: 32380762
85
Antimicrobial, cytotoxic, and insulin-releasing activities of the amphibian host-defense peptide ocellatin-3N and its L-lysine-substituted analogs.
Conlon JM, Hunter L, Attoub S, Casciaro B, Mechkarska M, Abdel-Wahab YHA. J Pept Sci. 2022 Nov 24:e3463. doi: 10.1002/psc.3463. Online ahead of print. PMID: 36426386

86
Seleno-vs. thioether triazine derivatives in search for new anticancer agents overcoming multidrug resistance in lymphoma.
Ali W, Garbo S, Kincses A, Nové M, Spengler G, Di Bello E, Honkisz-Orzechowska E, Karcz T, Szymańska E, Żesławska E, Starek M, Dąbrowska M, Nitek W, Kucwaj-Brysz K, Pyka P, Fioravanti R, Jacob C, Battistelli C, Zwergel C, Handzlik J. Eur J Med Chem. 2022 Dec 5;243:114761. doi: 10.1016/j.ejmech.2022.114761. Epub 2022 Sep 19. PMID: 36179403
87
DNA damage triggers an interplay between wtp53 and c-Myc affecting lymphoma cell proliferation and Kaposi sarcoma herpesvirus replication.
Arena A, Gilardini Montani MS, Romeo MA, Benedetti R, Gaeta A, Cirone M. Biochim Biophys Acta Mol Cell Res. 2022 Jan;1869(1):119168. doi: 10.1016/j.bbamcr.2021.119168. Epub 2021 Oct 30. PMID: 34728235
88
De novo transcriptome assembly and annotation of the third stage larvae of the zoonotic parasite Anisakis pegreffii.
Palomba M, Libro P, Di Martino J, Rughetti A, Santoro M, Mattiucci S, Castrignanò T. BMC Res Notes. 2022 Jun 25;15(1):223. doi: 10.1186/s13104-022-06099-9. PMID: 35752825
89
Restoration of WT1/miR-769-5p axis by HDAC1 inhibition promotes MMT reversal in mesenchymal-like mesothelial cells.
Bontempi G, Terri M, Garbo S, Montaldo C, Mariotti D, Bordoni V, Valente S, Zwergel C, Mai A, Marchetti A, Domenici A, Menè P, Battistelli C, Tripodi M, Strippoli R. Cell Death Dis. 2022 Nov 17;13(11):965. doi: 10.1038/s41419-022-05398-0. PMID: 36396626
90
Early Routine Biomarkers of SARS-CoV-2 Morbidity and Mortality: Outcomes from an Emergency Section.
Ceci FM, Fiore M, Gavaruzzi F, Angeloni A, Lucarelli M, Scagnolari C, Bonci E, Gabanella F, Di Certo MG, Barbato C, Petrella C, Greco A, Vincentiis M, Ralli M, Passananti C, Poscia R, Minni A, Ceccanti M, Tarani L, Ferraguti G. Diagnostics (Basel). 2022 Jan 12;12(1):176. doi: 10.3390/diagnostics12010176. PMID: 35054342
91
The dysregulation of autophagy and ER stress induced by HHV-6A infection activates pro-inflammatory pathways and promotes the release of inflammatory cytokines and cathepsin S by CNS cells.
Romeo MA, Gilardini Montani MS, Benedetti R, Arena A, Gaeta A, Cirone M. Virus Res. 2022 May;313:198726. doi: 10.1016/j.virusres.2022.198726. Epub 2022 Mar 4. PMID: 35248672

92
VPA and TSA Interrupt the Interplay between mutp53 and HSP70, Leading to CHK1 and RAD51 Down-Regulation and Sensitizing Pancreatic Cancer Cells to AZD2461 PARP Inhibitor.
Romeo MA, Gilardini Montani MS, Benedetti R, Arena A, D'Orazi G, Cirone M. Int J Mol Sci. 2022 Feb 18;23(4):2268. doi: 10.3390/ijms23042268. PMID: 35216385
93
ent-Beyerane Diterpenes as a Key Platform for the Development of ArnT-Mediated Colistin Resistance Inhibitors.
Quaglio D, Mangoni ML, Stefanelli R, Corradi S, Casciaro B, Vergine V, Lucantoni F, Cavinato L, Cammarone S, Loffredo MR, Cappiello F, Calcaterra A, Erazo S, Ghirga F, Mori M, Imperi F, Ascenzioni F, Botta B. J Org Chem. 2020 Aug 21;85(16):10891-10901. doi: 10.1021/acs.joc.0c01459. Epub 2020 Aug 10. PMID: 32806095
94
The impairment of DDR reduces XBP1s, further increasing DNA damage, and triggers autophagy via PERK/eIF2alpha in MM and IRE1alpha/JNK1/2 in PEL cells.
Arena A, Romeo MA, Benedetti R, Gilardini Montani MS, Cirone M. Biochem Biophys Res Commun. 2022 Jul 12;613:19-25. doi: 10.1016/j.bbrc.2022.04.108. Epub 2022 May 3. PMID: 35526484
95
Disclosing complex mutational dynamics at a Y chromosome palindrome evolving through intra- and inter-chromosomal gene conversion.
Bonito M, Ravasini F, Novelletto A, D'Atanasio E, Cruciani F, Trombetta B. Hum Mol Genet. 2022 Aug 3:ddac144. doi: 10.1093/hmg/ddac144. Online ahead of print. PMID: 35921243
96
Vitamin B6 rescues insulin resistance and glucose-induced DNA damage caused by reduced activity of Drosophila PI3K.
Mascolo E, Liguori F, Merigliano C, Schiano L, Gnocchini E, Pilesi E, Volonté C, Di Salvo ML, Contestabile R, Tramonti A, Vernì F. J Cell Physiol. 2022 Sep;237(9):3578-3586. doi: 10.1002/jcp.30812. Epub 2022 Jun 9. PMID: 35678366
97
Virus-like particle - mediated delivery of the RIG-I agonist M8 induces a type I interferon response and protects cells against viral infection.
Palermo E, Alexandridi M, Di Carlo D, Muscolini M, Hiscott J. Front Cell Infect Microbiol. 2022 Dec 14;12:1079926. doi: 10.3389/fcimb.2022.1079926. eCollection 2022. PMID: 36590581

98
The Triprenylated Anthranoid Ferruginin A, a Promising Scaffold for the Development of Novel Antibiotics against Gram-Positive Bacteria.
Casciaro B, Ghirga F, Cappiello F, Vergine V, Loffredo MR, Cammarone S, Puglisi E, Tortora C, Quaglio D, Mori M, Botta B, Mangoni ML. Antibiotics (Basel). 2022 Jan 11;11(1):84. doi: 10.3390/antibiotics11010084. PMID: 35052961
99
Characterization of the Escherichia coli pyridoxal 5'-phosphate homeostasis protein (YggS): Role of lysine residues in PLP binding and protein stability.
Tramonti A, Ghatge MS, Babor JT, Musayev FN, di Salvo ML, Barile A, Colotti G, Giorgi A, Paredes SD, Donkor AK, Al Mughram MH, de Crécy-Lagard V, Safo MK, Contestabile R. Protein Sci. 2022 Nov;31(11):e4471. doi: 10.1002/pro.4471. PMID: 36218140
100
Potential Use of Tea Tree Oil as a Disinfectant Agent against Coronaviruses: A Combined Experimental and Simulation Study.
Romeo A, Iacovelli F, Scagnolari C, Scordio M, Frasca F, Condò R, Ammendola S, Gaziano R, Anselmi M, Divizia M, Falconi M. Molecules. 2022 Jun 12;27(12):3786. doi: 10.3390/molecules27123786. PMID: 35744913
101
Increased heroin intake and relapse vulnerability in intermittent relative to continuous self-administration: Sex differences in rats.
D'Ottavio G, Reverte I, Ragozzino D, Meringolo M, Milella MS, Boix F, Venniro M, Badiani A, Caprioli D. Br J Pharmacol. 2022 Jan 5:10.1111/bph.15791. doi: 10.1111/bph.15791. Online ahead of print. PMID: 34986504
102
The Inhibition of DNA Viruses by the Amphibian Antimicrobial Peptide Temporin G: A Virological Study Addressing HSV-1 and JPCyV.
Marcocci ME, Jackowska BG, Prezioso C, Protto V, De Angelis M, Di Leva FS, Casciaro B, Carotenuto A, Mangoni ML, Palamara AT, Pietropaolo V, De Chiara G, Nencioni L. Int J Mol Sci. 2022 Jun 28;23(13):7194. doi: 10.3390/ijms23137194. PMID: 35806198
103
Adaptive strategies of uropathogenic Escherichia coli CFT073: from growth in lab media to virulence during host cell adhesion.
Sarshar M, Scribano D, Limongi D, Zagaglia C, Palamara AT, Ambrosi C. Int Microbiol. 2022 Aug;25(3):481-494. doi: 10.1007/s10123-022-00235-y. Epub 2022 Feb 2. PMID: 35106679

104
An Innovation 10 Years in the Making: The Stories in the Pages of ACS Medicinal Chemistry Letters.
Altman RA, Brai A, Golden J, La Regina G, Li Z, Moore TW, Pomerantz WCK, Rajapaksa NS, Adams AM. ACS Med Chem Lett. 2022 Feb 3;13(4):540-545. doi: 10.1021/acsmedchemlett.1c00623. eCollection 2022 Apr 14. PMID: 35450346
105
Author Correction: Low dose rate γ-irradiation protects fruit fly chromosomes from double strand breaks and telomere fusions by reducing the esi-RNA biogenesis factor Loquacious.
Porrazzo A, Cipressa F, De Gregorio A, De Pittà C, Sales G, Ciapponi L, Morciano P, Esposito G, Tabocchini MA, Cenci G. Commun Biol. 2022 Sep 29;5(1):1033. doi: 10.1038/s42003-022-03984-8. PMID: 36175493
106
First COVID-19 lockdown resulted in most respiratory viruses disappearing among hospitalised children, with the exception of rhinoviruses.
Nenna R, Matera L, Pierangeli A, Oliveto G, Viscido A, Petrarca L, La Regina DP, Mancino E, Di Mattia G, Villani A, Midulla F. Acta Paediatr. 2022 Jul;111(7):1399-1403. doi: 10.1111/apa.16326. Epub 2022 Mar 16. PMID: 35266576
107
Antifungal Activity of the Frog Skin Peptide Temporin G and Its Effect on Candida albicans Virulence Factors.
D'Auria FD, Casciaro B, De Angelis M, Marcocci ME, Palamara AT, Nencioni L, Mangoni ML. Int J Mol Sci. 2022 Jun 6;23(11):6345. doi: 10.3390/ijms23116345. PMID: 35683025
108
3,4-Dihydroxyphenylethanol (DPE or Hydroxytyrosol) Counteracts ERK1/2 and mTOR Activation, Pro-Inflammatory Cytokine Release, Autophagy and Mitophagy Reduction Mediated by Benzo[a]pyrene in Primary Human Colonic Epithelial Cells.
Santarelli R, Pompili C, Gilardini Montani MS, Evangelista L, Gonnella R, Cirone M. Pharmaceutics. 2022 Mar 17;14(3):663. doi: 10.3390/pharmaceutics14030663. PMID: 35336037
109
The Antimicrobial Peptide Esc(1-21) Synergizes with Colistin in Inhibiting the Growth and in Killing Multidrug Resistant Acinetobacter baumannii Strains.
Sacco F, Bitossi C, Casciaro B, Loffredo MR, Fabiano G, Torrini L, Raponi F, Raponi G, Mangoni ML. Antibiotics (Basel). 2022 Feb 11;11(2):234. doi: 10.3390/antibiotics11020234. PMID: 35203836

110
Low dose rate γ-irradiation protects fruit fly chromosomes from double strand breaks and telomere fusions by reducing the esi-RNA biogenesis factor Loquacious.
Porrazzo A, Cipressa F, De Gregorio A, De Pittà C, Sales G, Ciapponi L, Morciano P, Esposito G, Tabocchini MA, Cenci G. Commun Biol. 2022 Sep 3;5(1):905. doi: 10.1038/s42003-022-03885-w. PMID: 36057690
111
Is Côte D'Ivoire a new high hybridization zone for the two major malaria vectors, Anopheles coluzzii and An. gambiae (Diptera, Culicidae)?
Caputo B, Tondossoma N, Virgillito C, Pichler V, Serini P, Calzetta M, Manica M, Coulibaly ZI, Dia I, Akré M, Offianan A, Della Torre A. Infect Genet Evol. 2022 Mar;98:105215. doi: 10.1016/j.meegid.2022.105215. Epub 2022 Jan 19. PMID: 35063691
112
COS-7 and SVGp12 Cellular Models to Study JCPyV Replication and MicroRNA Expression after Infection with Archetypal and Rearranged-NCCR Viral Strains.
Prezioso C, Passerini S, Limongi D, Palamara AT, Moens U, Pietropaolo V. Viruses. 2022 Sep 17;14(9):2070. doi: 10.3390/v14092070. PMID: 36146876
113
The PDE4 inhibitor tanimilast shows distinct immunomodulatory properties associated with a type 2 endotype and CD141 upregulation.
Nguyen HO, Salvi V, Tiberio L, Facchinetti F, Govoni M, Villetti G, Civelli M, Barbazza I, Gaudenzi C, Passari M, Schioppa T, Sozio F, Del Prete A, Sozzani S, Bosisio D. J Transl Med. 2022 May 10;20(1):203. doi: 10.1186/s12967-022-03402-x. PMID: 35538539
114
Pulmonary Safety Profile of Esc Peptides and Esc-Peptide-Loaded Poly(lactide-co-glycolide) Nanoparticles: A Promising Therapeutic Approach for Local Treatment of Lung Infectious Diseases.
Cappiello F, Casciaro B, Loffredo MR, Puglisi E, Lin Q, Yang D, Conte G, d'Angelo I, Ungaro F, Ferrera L, Barbieri R, Cresti L, Pini A, Di YP, Mangoni ML. Pharmaceutics. 2022 Oct 26;14(11):2297. doi: 10.3390/pharmaceutics14112297. PMID: 36365116
115
High prevalence of Merkel cell polyomavirus is associated with dysregulation in transcript levels of TLR9 and type I IFNs in a large cohort of CF patients from the Italian (Lazio) reference center for cystic fibrosis.
Bitossi C, Viscido A, Prezioso C, Brazzini G, Trancassini M, Borrazzo C, Passerini S, Frasca F, Scordio M, Sorrentino L, Oliveto G, Fracella M, D'Auria A, Selvaggi C, Cimino G, Midulla F, Pierangeli A, Antonelli G, Moens U, Pietropaolo V, Scagnolari C. Microb Pathog. 2022 Aug;169:105644. doi: 10.1016/j.micpath.2022.105644. Epub 2022 Jun 22. PMID: 35752381

116
Nigritanine as a New Potential Antimicrobial Alkaloid for the Treatment of Staphylococcus aureus-Induced Infections.
Casciaro B, Calcaterra A, Cappiello F, Mori M, Loffredo MR, Ghirga F, Mangoni ML, Botta B, Quaglio D. Toxins (Basel). 2019 Sep 1;11(9):511. doi: 10.3390/toxins11090511. PMID: 31480508
117
The botanical drug PBI-05204, a supercritical CO2 extract of Nerium oleander, sensitizes alveolar and embryonal rhabdomyosarcoma to radiotherapy in vitro and in vivo.
Vaccaro S, Rossetti A, Porrazzo A, Camero S, Cassandri M, Pomella S, Tomaciello M, Macioce G, Pedini F, Barillari G, Marchese C, Rota R, Cenci G, Tombolini M, Newman RA, Yang P, Codenotti S, Fanzani A, Megiorni F, Festuccia C, Minniti G, Gravina GL, Vulcano F, Milazzo L, Marampon F. Front Pharmacol. 2022 Dec 1;13:1071176. doi: 10.3389/fphar.2022.1071176. eCollection 2022. PMID: 36532747
118
Diagnostic Value of JC Polyomavirus Viruria, Viremia, Serostatus and microRNA Expression in Multiple Sclerosis Patients Undergoing Immunosuppressive Treatment.
Prezioso C, Ciotti M, Brazzini G, Piacentini F, Passerini S, Grimaldi A, Landi D, Nicoletti CG, Zingaropoli MA, Iannetta M, Altieri M, Conte A, Limongi D, Marfia GA, Ciardi MR, Mastroianni CM, Palamara AT, Moens U, Pietropaolo V. J Clin Med. 2022 Jan 11;11(2):347. doi: 10.3390/jcm11020347. PMID: 35054041
119
Exposure to b-LED Light While Exerting Antimicrobial Activity on Gram-Negative and -Positive Bacteria Promotes Transient EMT-like Changes and Growth Arrest in Keratinocytes.
Terri M, Mancianti N, Trionfetti F, Casciaro B, de Turris V, Raponi G, Bontempi G, Montaldo C, Domenici A, Menè P, Mangoni ML, Strippoli R. Int J Mol Sci. 2022 Feb 8;23(3):1896. doi: 10.3390/ijms23031896. PMID: 35163819
120
Large-Scale Profiling of Extracellular Vesicles Identified miR-625-5p as a Novel Biomarker of Immunotherapy Response in Advanced Non-Small-Cell Lung Cancer Patients.
Pantano F, Zalfa F, Iuliani M, Simonetti S, Manca P, Napolitano A, Tiberi S, Russano M, Citarella F, Foderaro S, Vulpis E, Zingoni A, Masuelli L, Bei R, Ribelli G, Del Re M, Danesi R, Vincenzi B, Perrone G, Tonini G, Santini D. Cancers (Basel). 2022 May 14;14(10):2435. doi: 10.3390/cancers14102435. PMID: 35626040
121
Inhibition of Polycomb Repressive Complex 2 activity reduces trimethylation of H3K27 and affects development in Arabidopsis seedlings.
Ruta V, Longo C, Boccaccini A, Madia VN, Saccoliti F, Tudino V, Di Santo R, Lorrai R, Dello Ioio R, Sabatini S, Costi R, Costantino P, Vittorioso P. BMC Plant Biol. 2019 Oct 16;19(1):429. doi: 10.1186/s12870-019-2057-7. PMID: 31619182

122
1H-NMR metabolomics reveals the Glabrescione B exacerbation of glycolytic metabolism beside the cell growth inhibitory effect in glioma.
D'Alessandro G, Quaglio D, Monaco L, Lauro C, Ghirga F, Ingallina C, De Martino M, Fucile S, Porzia A, Di Castro MA, Bellato F, Mastrotto F, Mori M, Infante P, Turano P, Salmaso S, Caliceti P, Di Marcotullio L, Botta B, Ghini V, Limatola C. Cell Commun Signal. 2019 Aug 28;17(1):108. doi: 10.1186/s12964-019-0421-8. PMID: 31455353
123
Esc peptides as novel potentiators of defective cystic fibrosis transmembrane conductance regulator: an unprecedented property of antimicrobial peptides.
Ferrera L, Cappiello F, Loffredo MR, Puglisi E, Casciaro B, Botta B, Galietta LJV, Mori M, Mangoni ML. Cell Mol Life Sci. 2021 Dec 31;79(1):67. doi: 10.1007/s00018-021-04030-2. PMID: 34971429
124
A longitudinal study defined circulating microRNAs as reliable biomarkers for disease prognosis and progression in ALS human patients.
Dobrowolny G, Martone J, Lepore E, Casola I, Petrucci A, Inghilleri M, Morlando M, Colantoni A, Scicchitano BM, Calvo A, Bisogni G, Chiò A, Sabatelli M, Bozzoni I, Musarò A. Cell Death Discov. 2021 Jan 11;7(1):4. doi: 10.1038/s41420-020-00397-6. PMID: 33431881
125
Discovery of phenylselenoether-hydantoin hybrids as ABCB1 efflux pump modulating agents with cytotoxic and antiproliferative actions in resistant T-lymphoma.
Ali W, Spengler G, Kincses A, Nové M, Battistelli C, Latacz G, Starek M, Dąbrowska M, Honkisz-Orzechowska E, Romanelli A, Rasile MM, Szymańska E, Jacob C, Zwergel C, Handzlik J. Eur J Med Chem. 2020 Aug 15;200:112435. doi: 10.1016/j.ejmech.2020.112435. Epub 2020 May 15. PMID: 32505850
126
Modulating undruggable targets to overcome cancer therapy resistance.
Passirani C, Vessières A, La Regina G, Link W, Silvestri R. Drug Resist Updat. 2022 Jan;60:100788. doi: 10.1016/j.drup.2021.100788. Epub 2021 Dec 3. PMID: 35168144
127
Targeting the anti-apoptotic Bcl-2 family proteins: machine learning virtual screening and biological evaluation of new small molecules.
Valentini E, D'Aguanno S, Di Martile M, Montesano C, Ferraresi V, Patsilinakos A, Sabatino M, Antonini L, Chiacchiarini M, Valente S, Mai A, Colotti G, Ragno R, Trisciuoglio D, Del Bufalo D. Theranostics. 2022 Feb 28;12(5):2427-2444. doi: 10.7150/thno.64233. eCollection 2022. PMID: 35265218

128
Sirtuin modulators: past, present, and future perspectives.
Fiorentino F, Mautone N, Menna M, D'Acunzo F, Mai A, Rotili D. Future Med Chem. 2022 Jun;14(12):915-939. doi: 10.4155/fmc-2022-0031. Epub 2022 May 18. PMID: 35583203
129
PIGR-enriched circulating vesicles contributes to hepatocellular carcinoma aggressiveness.
Zingoni A, Banales JM. J Hepatol. 2022 Apr;76(4):768-770. doi: 10.1016/j.jhep.2022.02.001. Epub 2022 Feb 8. PMID: 35149124
130
New Insights Into the Role of GSK-3 in the Regulation of Human Cardiac Electrophysiology.
Forte M, Iacovone G, Sciarretta S. JACC Basic Transl Sci. 2022 Oct 24;7(10):1018-1020. doi: 10.1016/j.jacbts.2022.06.011. eCollection 2022 Oct. PMID: 36337925
131
PD-L1 small-molecule modulators: A new hope in epigenetic-based multidrug cancer therapy?
Zwergel C, Fioravanti R, Mai A. Drug Discov Today. 2022 Nov 9;28(2):103435. doi: 10.1016/j.drudis.2022.103435. Online ahead of print. PMID: 36370994
132
Therapeutic Potential and Activity Modulation of the Protein Lysine Deacylase Sirtuin 5.
Fiorentino F, Castiello C, Mai A, Rotili D. J Med Chem. 2022 Jul 28;65(14):9580-9606. doi: 10.1021/acs.jmedchem.2c00687. Epub 2022 Jul 8. PMID: 35802779
133
Targeting Chemokines and Chemokine GPCRs to Enhance Strong Opioid Efficacy in Neuropathic Pain.
Vincenzi M, Milella MS, D'Ottavio G, Caprioli D, Reverte I, Maftei D. Life (Basel). 2022 Mar 9;12(3):398. doi: 10.3390/life12030398. PMID: 35330149 Free PMC article.
134
Sex differences in the immune system: Implications for cocaine relapse.
Reverte I, D'Ottavio G, Milella MS, Caprioli D. Brain Behav Immun. 2022 May 14;104:29-30. doi: 10.1016/j.bbi.2022.05.009. Online ahead of print. PMID: 35580793
135
PNC2 (SLC25A36) Deficiency Associated With the Hyperinsulinism/Hyperammonemia Syndrome.
Shahroor MA, Lasorsa FM, Porcelli V, Dweikat I, Di Noia MA, Gur M, Agostino G, Shaag A, Rinaldi T, Gasparre G, Guerra F, Castegna A, Todisco S, Abu-Libdeh B, Elpeleg O, Palmieri L. J Clin Endocrinol Metab. 2022 Apr 19;107(5):1346-1356. doi: 10.1210/clinem/dgab932. PMID: 34971397

136
The Acinetobacter baumannii model can explain the role of small non-coding RNAs as potential mediators of host-pathogen interactions.
Sarshar M, Scribano D, Palamara AT, Ambrosi C, Masotti A. Front Mol Biosci. 2022 Dec 21;9:1088783. doi: 10.3389/fmolb.2022.1088783. eCollection 2022. PMID: 36619166
137
Anticancer Activity of (S)-5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(1-oxo-1-((pyridin-4-ylmethyl)amino)propan-2-yl)-1H-indole-2-carboxamide (RS4690), a New Dishevelled 1 Inhibitor.
Coluccia A, Bufano M, La Regina G, Puxeddu M, Toto A, Paone A, Bouzidi A, Musto G, Badolati N, Orlando V, Biagioni S, Masci D, Cantatore C, Cirilli R, Cutruzzolà F, Gianni S, Stornaiuolo M, Silvestri R. Cancers (Basel). 2022 Mar 7;14(5):1358. doi: 10.3390/cancers14051358. PMID: 35267666 Free PMC article.
Compound (S)-1 inhibited the growth of HCT116 cells expressing wild-type APC with an EC(50) of 7.1 0.6 muM and caused a high level of ROS production. These results highlight (S)-1 as a lead compound for the development of new therapeutic agents against WNT-dependent …
138
Chemically Diverse S. mansoni HDAC8 Inhibitors Reduce Viability in Worm Larval and Adult Stages.
Noce B, Di Bello E, Zwergel C, Fioravanti R, Valente S, Rotili D, Masotti A, Salik Zeya Ansari M, Trisciuoglio D, Chakrabarti A, Romier C, Robaa D, Sippl W, Jung M, Häberli C, Keiser J, Mai A. ChemMedChem. 2022 Oct 17:e202200510. doi: 10.1002/cmdc.202200510. Online ahead of print. PMID: 36250286
139
Clinical outcomes of a large cohort of individuals with the F508del/5T;TG12 CFTR genotype.
Tosco A, Castaldo A, Colombo C, Claut L, Carnovale V, Iacotucci P, Lucarelli M, Cimino G, Fabrizzi B, Caporelli N, Majo F, Ciciriello F, Padoan R, Poli P, Taccetti G, Centrone C, Casciaro R, Castellani C, Salvatore D, Colangelo C, Bonomi P, Castaldo G, Terlizzi V. J Cyst Fibros. 2022 Sep;21(5):850-855. doi: 10.1016/j.jcf.2022.04.020. Epub 2022 May 4. PMID: 35523714
140
Correction: Identification of a novel quinoline-based DNA demethylating compound highly potent in cancer cells.
Zwergel C, Schnekenburger M, Sarno F, Battistelli C, Manara MC, Stazi G, Mazzone R, Fioravanti R, Gros C, Ausseil F, Florean C, Nebbioso A, Strippoli R, Ushijima T, Scotlandi K, Tripodi M, Arimondo PB, Altucci L, Diederich M, Mai A, Valente S. Clin Epigenetics. 2022 Sep 28;14(1):119. doi: 10.1186/s13148-022-01339-y. PMID: 36171618

141
Inflammatory Response in Caco-2 Cells Stimulated with Anisakis Messengers of Pathogenicity.
Bellini I, Scribano D, Sarshar M, Ambrosi C, Pizzarelli A, Palamara AT, D'Amelio S, Cavallero S. Pathogens. 2022 Oct 20;11(10):1214. doi: 10.3390/pathogens11101214. PMID: 36297271
142
Discovery of novel human lactate dehydrogenase inhibitors: Structure-based virtual screening studies and biological assessment.
Di Magno L, Coluccia A, Bufano M, Ripa S, La Regina G, Nalli M, Di Pastena F, Canettieri G, Silvestri R, Frati L. Eur J Med Chem. 2022 Oct 5;240:114605. doi: 10.1016/j.ejmech.2022.114605. Epub 2022 Jul 14. PMID: 35868126
143
Effects of Structurally Different HDAC Inhibitors against Trypanosoma cruzi, Leishmania, and Schistosoma mansoni.
Di Bello E, Noce B, Fioravanti R, Zwergel C, Valente S, Rotili D, Fianco G, Trisciuoglio D, Mourão MM, Sales P Jr, Lamotte S, Prina E, Späth GF, Häberli C, Keiser J, Mai A. ACS Infect Dis. 2022 Jul 8;8(7):1356-1366. doi: 10.1021/acsinfecdis.2c00232. Epub 2022 Jun 22. PMID: 35732073
144
Novel non-covalent LSD1 inhibitors endowed with anticancer effects in leukemia and solid tumor cellular models.
Menna M, Fiorentino F, Marrocco B, Lucidi A, Tomassi S, Cilli D, Romanenghi M, Cassandri M, Pomella S, Pezzella M, Del Bufalo D, Zeya Ansari MS, Tomašević N, Mladenović M, Viviano M, Sbardella G, Rota R, Trisciuoglio D, Minucci S, Mattevi A, Rotili D, Mai A. Eur J Med Chem. 2022 Jul 5;237:114410. doi: 10.1016/j.ejmech.2022.114410. Epub 2022 Apr 27. PMID: 35525212
145
Analysis of serum microRNAs and rs2910164 GC single-nucleotide polymorphism of miRNA-146a in COVID-19 patients.
Pinacchio C, Scordio M, Santinelli L, Frasca F, Sorrentino L, Bitossi C, Oliveto G, Viscido A, Ceci FM, Celani L, Ceccarelli G, Antonelli G, Mastroianni CM, d'Ettorre G, Scagnolari C. J Immunoassay Immunochem. 2022 Jul 4;43(4):347-364. doi: 10.1080/15321819.2022.2035394. Epub 2022 Feb 28. PMID: 35227165
146
Downregulation of epithelial sodium channel (ENaC) activity in cystic fibrosis cells by epigenetic targeting.
Blaconà G, Raso R, Castellani S, Pierandrei S, Del Porto P, Ferraguti G, Ascenzioni F, Conese M, Lucarelli M. Cell Mol Life Sci. 2022 Apr 25;79(5):257. doi: 10.1007/s00018-022-04190-9. PMID: 35462606

147
Multiplexed cellular profiling identifies an organoselenium compound as an inhibitor of CRM1-mediated nuclear export.
Jimenez L, Mayoral-Varo V, Amenábar C, Ortega J, Sequeira JGN, Machuqueiro M, Mourato C, Silvestri R, Angeli A, Carta F, Supuran CT, Megías D, Ferreira BI, Link W. Traffic. 2022 Dec;23(12):587-599. doi: 10.1111/tra.12872. PMID: 36353954
148
Effects of 3,4-diaminopyridine on myasthenia gravis: Preliminary results of an open-label study.
Ceccanti M, Libonati L, Ruffolo G, Cifelli P, Moret F, Frasca V, Palma E, Inghilleri M, Cambieri C. Front Pharmacol. 2022 Aug 16;13:982434. doi: 10.3389/fphar.2022.982434. eCollection 2022. PMID: 36052140
149
Printed Electrochemical Strip for the Detection of miRNA-29a: A Possible Biomarker Related to Alzheimer's Disease.
Miglione A, Raucci A, Amato J, Marzano S, Pagano B, Raia T, Lucarelli M, Fuso A, Cinti S. Anal Chem. 2022 Nov 15;94(45):15558-15563. doi: 10.1021/acs.analchem.2c03542. Epub 2022 Nov 1. PMID: 36318963
150
Bovine Serum Amine Oxidase and Polyamine Analogues: Chemical Synthesis and Biological Evaluation Integrated with Molecular Docking and 3-D QSAR Studies.
Ragno R, Minarini A, Proia E, Lorenzo A, Milelli A, Tumiatti V, Fiore M, Fino P, Rutigliano L, Fioravanti R, Tahara T, Pacella E, Greco A, Canettieri G, Di Paolo ML, Agostinelli E. J Chem Inf Model. 2022 Aug 22;62(16):3910-3927. doi: 10.1021/acs.jcim.2c00559. Epub 2022 Aug 10. PMID: 35948439
151
The Impact of NRF2 Inhibition on Drug-Induced Colon Cancer Cell Death and p53 Activity: A Pilot Study.
Garufi A, Pistritto G, D'Orazi V, Cirone M, D'Orazi G. Biomolecules. 2022 Mar 17;12(3):461. doi: 10.3390/biom12030461. PMID: 35327653
152
Novel pyridine-containing histone deacetylase inhibitors strongly arrest proliferation, induce apoptosis and modulate miRNAs in cancer cells.
Di Bello E, Sian V, Bontempi G, Zwergel C, Fioravanti R, Noce B, Castiello C, Tomassi S, Corinti D, Passeri D, Pellicciari R, Mercurio C, Varasi M, Altucci L, Tripodi M, Strippoli R, Nebbioso A, Valente S, Mai A. Eur J Med Chem. 2022 Dec 15;247:115022. doi: 10.1016/j.ejmech.2022.115022. Online ahead of print. PMID: 36549114

153
Opposite Effect of Thyroid Hormones on Oxidative Stress and on Mitochondrial Respiration in COVID-19 Patients.
De Vitis C, Capalbo C, Torsello A, Napoli C, Salvati V, Loffredo C, Blandino G, Piaggio G, Auciello FR, Pelliccia F, Salerno G, Simmaco M, Di Magno L, Canettieri G, Coluzzi F, Mancini R, Rocco M, Sciacchitano S. Antioxidants (Basel). 2022 Oct 8;11(10):1998. doi: 10.3390/antiox11101998. PMID: 36290721
154
Systematic Design of Adenosine Analogs as Inhibitors of a Clostridioides difficile-Specific DNA Adenine Methyltransferase Required for Normal Sporulation and Persistence.
Zhou J, Horton JR, Menna M, Fiorentino F, Ren R, Yu D, Hajian T, Vedadi M, Mazzoccanti G, Ciogli A, Weinhold E, Hüben M, Blumenthal RM, Zhang X, Mai A, Rotili D, Cheng X. J Med Chem. 2023 Jan 12;66(1):934-950. doi: 10.1021/acs.jmedchem.2c01789. Epub 2022 Dec 29. PMID: 36581322
155
Polymerogenic neuroserpin causes mitochondrial alterations and activates NFκB but not the UPR in a neuronal model of neurodegeneration FENIB.
D'Acunto E, Gianfrancesco L, Serangeli I, D'Orsi M, Sabato V, Guadagno NA, Bhosale G, Caristi S, Failla AV, De Jaco A, Cacci E, Duchen MR, Lupo G, Galliciotti G, Miranda E. Cell Mol Life Sci. 2022 Jul 21;79(8):437. doi: 10.1007/s00018-022-04463-3. PMID: 35864382
156
Alzheimer's Disease with Epileptiform EEG Activity: Abnormal Cortical Sources of Resting State Delta Rhythms in Patients with Amnesic Mild Cognitive Impairment.
Babiloni C, Noce G, Di Bonaventura C, Lizio R, Eldellaa A, Tucci F, Salamone EM, Ferri R, Soricelli A, Nobili F, Famà F, Arnaldi D, Palma E, Cifelli P, Marizzoni M, Stocchi F, Bruno G, Di Gennaro G, Frisoni GB, Del Percio C. J Alzheimers Dis. 2022;88(3):903-931. doi: 10.3233/JAD-220442. PMID: 35694930
157
Comparative evaluation of molecular methods for the quantitative measure of torquetenovirus viremia, the new surrogate marker of immune competence.
Macera L, Spezia PG, Medici C, Rofi E, Del Re M, Focosi D, Mazzetti P, Navarro D, Antonelli G, Danesi R, Pistello M, Maggi F. J Med Virol. 2022 Feb;94(2):491-498. doi: 10.1002/jmv.25488. Epub 2019 May 1. PMID: 31002743
158
RS6077 induces mitotic arrest and selectively activates cell death in human cancer cell lines and in a lymphoma tumor in vivo.
Sebastiani J, Puxeddu M, Nalli M, Bai R, Altieri L, Rovella P, Gaudio E, Trisciuoglio D, Spriano F, Lavia P, Fionda C, Masci D, Urbani A, Bigogno C, Dondio G, Hamel E, Bertoni F, Silvestri R, La Regina G. Eur J Med Chem. 2023 Jan 15;246:114997. doi: 10.1016/j.ejmech.2022.114997. Epub 2022 Dec 5. PMID: 36502578

159
Allostery Frustrates the Experimentalist.
Gianni S, Jemth P.
J Mol Biol. 2022 Dec 28;435(4):167934. doi: 10.1016/j.jmb.2022.167934. Online ahead of print.
PMID: 36586463
160
ERAP1 as an emerging therapeutic target for medulloblastoma.
Bufalieri F, Fruci D, Di Marcotullio L.
Trends Cancer. 2022 Jan;8(1):4-8. doi: 10.1016/j.trecan.2021.09.005. Epub 2021 Oct 20.
PMID: 34686465
161
SH2 Domains: Folding, Binding and Therapeutical Approaches.
Diop A, Santorelli D, Malagrinò F, Nardella C, Pennacchietti V, Pagano L, Marcocci L, Pietrangeli P, Gianni S, Toto A.
Int J Mol Sci. 2022 Dec 15;23(24):15944. doi: 10.3390/ijms232415944.
PMID: 36555586
162
An Outlook on the Complexity of Protein Morphogenesis in Health and Disease.
Brunori M, Gianni S.
Front Mol Biosci. 2022 Jun 13;9:909567. doi: 10.3389/fmolb.2022.909567. eCollection 2022.
PMID: 35769915
163
Editorial: Intrinsically Disordered Proteins and Regions: The Challenge to the Structure-Function Relationship.
Toto A, Sormanni P, Paissoni C, Uversky VN.
Front Mol Biosci. 2022 Jul 6;9:962643. doi: 10.3389/fmolb.2022.962643. eCollection 2022.
PMID: 35874612
164
The Mechanism of Action of Biguanides: New Answers to a Complex Question.
Di Magno L, Di Pastena F, Bordone R, Coni S, Canettieri G.
Cancers (Basel). 2022 Jun 30;14(13):3220. doi: 10.3390/cancers14133220.
PMID: 35804992
165
Classical and Unexpected Effects of Ultra-Micronized PEA in Neuromuscular Function.
Cifelli P, Ruffolo G, Ceccanti M, Cambieri C, Libonati L, Palma E, Inghilleri M.
Biomolecules. 2022 May 29;12(6):758. doi: 10.3390/biom12060758.
PMID: 35740883

166
Hedgehog signaling pathway inhibitors: an updated patent review (2015-present).
Quaglio D, Infante P, Di Marcotullio L, Botta B, Mori M.
Expert Opin Ther Pat. 2020 Apr;30(4):235-250. doi: 10.1080/13543776.2020.1730327. Epub 2020 Feb 19.
PMID: 32070165
167
Proteolysis-Targeting Chimera (PROTAC): Is the Technology Looking at the Treatment of Brain Tumors?
Lospinoso Severini L, Bufalieri F, Infante P, Di Marcotullio L.
Front Cell Dev Biol. 2022 Feb 15;10:854352. doi: 10.3389/fcell.2022.854352. eCollection 2022.
PMID: 35242765
168
Age-dependent NK cell dysfunctions in severe COVID-19 patients.
Fionda C, Ruggeri S, Sciumè G, Laffranchi M, Quinti I, Milito C, Palange P, Menichini I, Sozzani S, Frati L, Gismondi A, Santoni A, Stabile H.
Front Immunol. 2022 Nov 17;13:1039120. doi: 10.3389/fimmu.2022.1039120. eCollection 2022.
PMID: 36466890
169
Folding and Binding Mechanisms of the SH2 Domain from Crkl.
Nardella C, Toto A, Santorelli D, Pagano L, Diop A, Pennacchietti V, Pietrangeli P, Marcocci L, Malagrinò F, Gianni S.
Biomolecules. 2022 Jul 22;12(8):1014. doi: 10.3390/biom12081014.
PMID: 35892324
170
Acetylcholinesterase inhibitors for the treatment of Alzheimer's disease - a patent review (2016-present).
Bortolami M, Rocco D, Messore A, Di Santo R, Costi R, Madia VN, Scipione L, Pandolfi F.
Expert Opin Ther Pat. 2021 May;31(5):399-420. doi: 10.1080/13543776.2021.1874344. Epub 2021 Jan 14.
PMID: 33428491
171
On the Effects of Disordered Tails, Supertertiary Structure and Quinary Interactions on the Folding and Function of Protein Domains.
Malagrinò F, Pennacchietti V, Santorelli D, Pagano L, Nardella C, Diop A, Toto A, Gianni S.
Biomolecules. 2022 Jan 26;12(2):209. doi: 10.3390/biom12020209.
PMID: 35204709

172
Olfactory impairment in autoimmune encephalitis: another piece of the puzzle.
Morano A, Cerulli Irelli E, Fanella M, Orlando B, Salamone EM, Tinelli E, Ruffolo G, Zuliani L, Fattouch J, Manfredi M, Giallonardo AT, Di Bonaventura C.
J Neurol. 2022 May;269(5):2762-2768. doi: 10.1007/s00415-022-10959-6. Epub 2022 Jan 10.
PMID: 35006386
173
GABAergic Neurotransmission in Human Tissues Is Modulated by Cannabidiol.
Ruffolo G, Gaeta A, Cannata B, Pinzaglia C, Aronica E, Morano A, Cifelli P, Palma E.
Life (Basel). 2022 Dec 6;12(12):2042. doi: 10.3390/life12122042.
PMID: 36556407
174
Exploring the effect of tethered domains on the folding of Grb2 protein.
Pagano L, Pennacchietti V, Diop A, Santorelli D, Pietrangeli P, Marcocci L, Nardella C, Malagrinò F, Toto A, Gianni S.
Arch Biochem Biophys. 2022 Nov 30;731:109444. doi: 10.1016/j.abb.2022.109444. Epub 2022 Oct 18.
PMID: 36265650
175
GAS6/TAM signaling pathway controls MICA expression in multiple myeloma cells.
Kosta A, Mekhloufi A, Lucantonio L, Zingoni A, Soriani A, Cippitelli M, Gismondi A, Fazio F, Petrucci MT, Santoni A, Stabile H, Fionda C.
Front Immunol. 2022 Jul 28;13:942640. doi: 10.3389/fimmu.2022.942640. eCollection 2022.
PMID: 35967396
176
Late-onset seizures and epilepsy: Electroclinical features suggestive of autoimmune etiology.
Morano A, Cerulli Irelli E, Salamone EM, Orlando B, Fanella M, Tinelli E, Ruffolo G, Zuliani L, Fattouch J, Manfredi M, Giallonardo AT, Di Bonaventura C.
Front Neurol. 2022 Aug 12;13:924859. doi: 10.3389/fneur.2022.924859. eCollection 2022.
PMID: 36034285
177
A short structural extension dictates the early stages of folding of a PDZ domain.
Gautier C, Gianni S.
Biochim Biophys Acta Proteins Proteom. 2022 Nov 1;1870(11-12):140852. doi: 10.1016/j.bbapap.2022.140852. Epub 2022 Aug 31.
PMID: 36055518

178
Cryptic binding properties of a transient folding intermediate in a PDZ tandem repeat.
Malagrinò F, Fusco G, Pennacchietti V, Toto A, Nardella C, Pagano L, de Simone A, Gianni S.
Protein Sci. 2022 Sep;31(9):e4396. doi: 10.1002/pro.4396.
PMID: 36040267
179
Characterization of early and late transition states of the folding pathway of a SH2 domain.
Toto A, Malagrinò F, Nardella C, Pennacchietti V, Pagano L, Santorelli D, Diop A, Gianni S.
Protein Sci. 2022 Jun;31(6):e4332. doi: 10.1002/pro.4332.
PMID: 35634781
180
Ultrastructural Damages to H1N1 Influenza Virus Caused by Vapor Essential Oils.
Madia VN, Toscanelli W, De Vita D, De Angelis M, Messore A, Ialongo D, Scipione L, Tudino V, D'Auria FD, Di Santo R, Garzoli S, Stringaro A, Colone M, Marchetti M, Superti F, Nencioni L, Costi R.
Molecules. 2022 Jun 9;27(12):3718. doi: 10.3390/molecules27123718.
PMID: 35744845
181
Unexpected Effect of IL-1β on the Function of GABAA Receptors in Pediatric Focal Cortical Dysplasia.
Alfano V, Romagnolo A, Mills JD, Cifelli P, Gaeta A, Morano A, Mühlebner A, Aronica E, Palma E, Ruffolo G.
Brain Sci. 2022 Jun 19;12(6):807. doi: 10.3390/brainsci12060807.
PMID: 35741692
182
ATF6 prevents DNA damage and cell death in colon cancer cells undergoing ER stress.
Benedetti R, Romeo MA, Arena A, Gilardini Montani MS, Di Renzo L, D'Orazi G, Cirone M.
Cell Death Discov. 2022 Jun 25;8(1):295. doi: 10.1038/s41420-022-01085-3.
PMID: 35752616
183
In Vitro Antiviral Activity of New Oxazoline Derivatives as Potent Poliovirus Inhibitors.
Madia VN, Messore A, Pescatori L, Saccoliti F, Tudino V, De Leo A, Scipione L, Fiore L, Rhoden E, Manetti F, Oberste MS, Di Santo R, Costi R.
J Med Chem. 2019 Jan 24;62(2):798-810. doi: 10.1021/acs.jmedchem.8b01482. Epub 2018 Dec 18.
PMID: 30512950

184
Increased heroin intake and relapse vulnerability in intermittent relative to continuous self-administration: Sex differences in rats.
D'Ottavio G, Reverte I, Ragozzino D, Meringolo M, Milella MS, Boix F, Venniro M, Badiani A, Caprioli D.
Br J Pharmacol. 2022 Jan 5:10.1111/bph.15791. doi: 10.1111/bph.15791. Online ahead of print.
PMID: 34986504
185
Novel Targeting of DNA Methyltransferase Activity Inhibits Ewing Sarcoma Cell Proliferation and Enhances Tumor Cell Sensitivity to DNA Damaging Drugs by Activating the DNA Damage Response.
Cristalli C, Manara MC, Valente S, Pellegrini E, Bavelloni A, De Feo A, Blalock W, Di Bello E, Piñeyro D, Merkel A, Esteller M, Tirado OM, Mai A, Scotlandi K.
Front Endocrinol (Lausanne). 2022 May 31;13:876602. doi: 10.3389/fendo.2022.876602. eCollection 2022.
PMID: 35712255
186
The Inhibition of DNA Viruses by the Amphibian Antimicrobial Peptide Temporin G: A Virological Study Addressing HSV-1 and JPCyV.
Marcocci ME, Jackowska BG, Prezioso C, Protto V, De Angelis M, Di Leva FS, Casciaro B, Carotenuto A, Mangoni ML, Palamara AT, Pietropaolo V, De Chiara G, Nencioni L.
Int J Mol Sci. 2022 Jun 28;23(13):7194. doi: 10.3390/ijms23137194.
PMID: 35806198
187
An integrative in-silico analysis discloses a novel molecular subset of colorectal cancer possibly eligible for immune checkpoint immunotherapy.
Sibilio P, Belardinilli F, Licursi V, Paci P, Giannini G.
Biol Direct. 2022 May 9;17(1):10. doi: 10.1186/s13062-022-00324-y.
PMID: 35534873
188
GABAA receptor function is enhanced by Interleukin-10 in human epileptogenic gangliogliomas and its effect is counteracted by Interleukin-1β.
Ruffolo G, Alfano V, Romagnolo A, Zimmer T, Mills JD, Cifelli P, Gaeta A, Morano A, Anink J, Mühlebner A, Vezzani A, Aronica E, Palma E.
Sci Rep. 2022 Oct 26;12(1):17956. doi: 10.1038/s41598-022-22806-9.
PMID: 36289354

189
A gene dosage-dependent effect unveils NBS1 as both a haploinsufficient tumour suppressor and an essential gene for SHH-medulloblastoma.
Petroni M, Fabretti F, Di Giulio S, Nicolis di Robilant V, La Monica V, Moretti M, Belardinilli F, Bufalieri F, Coppa A, Paci P, Corsi A, De Smaele E, Coni S, Canettieri G, Di Marcotullio L, Wang ZQ, Giannini G.
Neuropathol Appl Neurobiol. 2022 Oct;48(6):e12837. doi: 10.1111/nan.12837. Epub 2022 Aug 10.PMID: 35839783
190
Manual versus automatic analysis of microglial density and distribution: a comparison in the hippocampus of healthy and lipopolysaccharide-challenged mature male mice.
Khakpour M, Ibáñez FG, Bordeleau M, Picard K, Mckee-Reid L, Ben-Azu B, Maggi L, Tremblay MÈ.
Micron. 2022 Oct;161:103334. doi: 10.1016/j.micron.2022.103334. Epub 2022 Aug 2.
PMID: 35970079
191
Lipid mediator n-3 docosapentaenoic acid-derived protectin D1 enhances synaptic inhibition of hippocampal principal neurons by interaction with a G-protein-coupled receptor.
Mikroulis A, Ledri M, Ruffolo G, Palma E, Sperk G, Dalli J, Vezzani A, Kokaia M.
FASEB J. 2022 Mar;36(3):e22203. doi: 10.1096/fj.202101815R.PMID: 35188290
192
The kinesin Eg5 inhibitor K858 exerts antiproliferative and proapoptotic effects and attenuates the invasive potential of head and neck squamous carcinoma cells.
Nicolai A, Taurone S, Carradori S, Artico M, Greco A, Costi R, Scarpa S.
Invest New Drugs. 2022 Jun;40(3):556-564. doi: 10.1007/s10637-022-01238-2. Epub 2022 Mar 21. PMID : 35312942
1953
Inhibition of Polycomb Repressive Complex 2 activity reduces trimethylation of H3K27 and affects development in Arabidopsis seedlings.
Ruta V, Longo C, Boccaccini A, Madia VN, Saccoliti F, Tudino V, Di Santo R, Lorrai R, Dello Ioio R, Sabatini S, Costi R, Costantino P, Vittorioso P.
BMC Plant Biol. 2019 Oct 16;19(1):429. doi: 10.1186/s12870-019-2057-7.
PMID: 31619182
194
Memory $CD8^+$ T cell diversity and B cell responses correlate with protection against SARS-CoV-2 following mRNA vaccination
Nadia Brasu [# 1 2], Ines Elia [# 1 3], Valentina Russo [# 1 2], Gaia Montacchiesi [# 1 2], Simona Aversano Stabile [# 1 3], Carlo De Intinis [1 3], Francesco Fesi [3], Katiuscia Gizzi [1 3], Marco Macagno [3], Monica Montone [3], Benedetta Mussolin [3], Alba Grifoni [4], Silvia Faravelli [5], Silvia Marchese [6], Federico Forneris [5], Raffaele De Francesco [6 7], Alessandro Sette [4 8], Vincenzo

Barnaba [9, 10], Antonino Sottile [3], Anna Sapino [3, 11], Luigia Pace [12, 13]DOI: 10.1038/s41590-022-01313-z

ADMINISTRATIVE BOARD

The Board of Administration is chaired by a President.
President
Luigi Frati
Members
Angela Santoni (Scientific Director), **Vincenzo Barba**, **Roberto Bruzzone, Giuseppe Sancetta**
Administrative Secretary
Maria Pia Lorenzoni
Auditors
Carla Vassallo, Adriana Vittazzi

SCIENTIFIC BOARD

The Scientific Council is a board of scientists active in the field of the pasteurian sciences.
Scientific Director
Angela Santoni (Immunology)
Members
Bianca Colonna (Microbiology), **Francesca Cutruzzolà** (Molecular Biology), **Stefano Gianni** (Biochemistry), **Giuseppe La Regina** (Novel therapeutic interventions), **Cristina Limatola** (Neurosciences), **Antonio Musarò** (Genetics, biology and pathophysiology of eukaryotes), **Anna Teresa Palamara** (Microbiology and Infectious Diseases), **Sergio Pimpinelli** (Genetics), **Sebastiano Sciarretta** (Novel therapeutic interventions), **Silvano Sozzani**(Immunology)

STAFF

Sara Atzeni, Elisabetta Canigiani, Francesca Lattanzi, **Maria Pia Lorenzoni**, **Lynda Romani**

ISTITUTO PASTEUR ITALIA – FONDAZIONE CENCI BOLOGNETTI
Viale Regina Elena 291 – 00161 – Rome
Tel. 0039 06 49255625
mail: pasteuritalia@istitutopasteuritalia.it
www.istitutopasteuritalia.it