A BIOMETRA BESTSELLER

THE COVID-19 PAPERS

BOOK OF ABSTRACTS

Breaking the story

of how during one of the worst

periods of our lives

we found the time for writing

down our own story

Sept. 28 2020, on the Internet

9:30: Welcome and Introduction
9:45: session I
Massimo Aureli - *GM1 as adjuvant of innovative therapies for Cystic Fibrosis disease*
Pietro Emiliano Doneddu & Eduardo Nobile-Orazio - *Acute and chronic Immune mediated neuropathies during COVID-19: associations and problems*
Daniela Lucini - *STAY HOME STAY FIT*
Angelisa Frasca - *In vivo magnetic resonance spectroscopy in the brain of Cdkl5 null mice reveals a metabolic profile indicative of mitochondrial dysfunctions*
Alessandra Longaretti & Francesco Rusconi - *Forgetting COVID-19, how mechanisms of memory inhibition help neutralizing traumatic stressful event*
11:00 break

Registration: https://forms.gle/LrQnnhgG2UqxgftR7
Info: workshop.biometra@unimi.it

11:30: session II
Federica Marchesi - *Macrophage morphology correlates with single-cell diversity and prognosis in colorectal liver metastasis*
Giuliano Zanchetta - *Kicking and breaking at the microscale: investigation of the yielding of soft materials with an optofluidic micro-rheometer*
Marco Cafora & Nicoletta Loberto - *Potential action of phages as immunomodulators in cystic fibrosis*
Samuele Ciceri - *The sad story of an enzymatic reaction in the fridge*
Francesca Calcaterra - *Flow cytometric characterization and transcriptomic profiling of dendritic cell subsets in high-grade glioma patients*
12:45: Closing Address

Presenting Authors

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2. Buscaglia M.
3. Cafora M.& Loberto N.
4. Calcaterra F.
5. Carenza C.
6. Chiricozzi E.
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GM1 as adjuvant of innovative therapies for Cystic Fibrosis disease

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein is expressed at the apical plasma membrane (PM) of different epithelial cells. The most common mutation responsible for the onset of cystic fibrosis (CF), F508del, inhibits biosynthesis and transport of the protein at PM and also presents gating and stability defects of membrane anion channel upon its rescue by the use of correctors and potentiators. This prompted a multiple drug strategy for F508delCFTR aimed in parallel on its rescue, functional potentiation and PM stabilization. Since ganglioside GM1 is involved in functional stabilization of transmembrane proteins, we investigated here its role as adjuvant to increase the effectiveness of CFTR modulators. According to our results, we found that GM1 resides in the same PM microenvironment of CFTR. In CF cells the expression of mutated channel is paralleled by a decrease in the PM GM1 content. Interestingly, by the exogenous administration of GM1, it becomes component of the PM and reduces the destabilizing effect of the potentiator VX-770 on rescued CFTR protein expression/function and improves its stabilization. This evidence could represent a starting point for developing innovative therapeutic strategies based on co-administration of GM1, correctors and potentiators, aimed to achieve an increased F508del CFTR function.

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Microfluidic lectins array for on-line bacteria detection

Rapid detection and quantification of molecular and microbiological contaminants in water samples are common needs in environmental monitoring applications. In this work we report a novel method enabling the detection and screening of bacteria strains in a microfluidic cell under continuous flow. Bacteria are captured by an array of different lectins on the surface of a Reflective Phantom Interface (RPI) biosensor prepared via direct or DNA-directed immobilization. A custom, portable optical set-up enables combining molecular and bacteria detection on the same sensor. The spots of immobilized lectins are characterized by RPI imaging in the blue, whereas the presence of bacteria and their binding is observed via dark field illumination in the yellow. By tracking the bacteria position in time under flow, the binding events on specific lectins spots are enumerated. We show that the spots prepared by DNA-directed immobilization of Griffonia Simplicifolia Lectin I provide the largest capturing capability for E.cloacae. The proposed method provides a novel tool to develop arrays for rapid screening of bacteria in water samples.

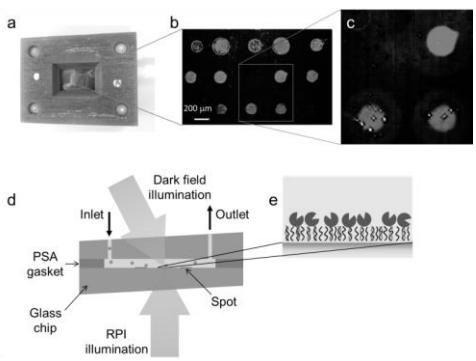

Figure 1. Fluidic cell hosting the lectin array for bacteria detection. (a) Image of the fluidic cell (b) and of the spotted RPI sensing surface. (c) Enlarged view of four streptavidin spots with bound nanoparticles imaged as yellow dots, used to test the detection system. (d) Schematic representation of the fluidic cell (e) and of the RPI surface spotted with lectins immobilized via DNA tethers.

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Potential action of phages as immunomodulators in cystic fibrosis

Chronic inflammation caused by bacterial infections is a common feature of patients with Cystic Fibrosis (CF). However, new evidence suggest that constitutive inflammation is present in CF patients even in the absence of bacterial infection. Surprisingly, we also found that phages mitigate the hyper-inflammation of the CF zebrafish model not infected by bacteria. Then, we investigated the mechanism through which phages act as anti-inflammatory agents using the CF zebrafish model and primary and immortalized human bronchial epithelial cells homozygous for the CFTR mutation F508del. In the zebrafish embryos we characterized the immunomodulatory effects of the phage-cocktail used in our preliminary work. We demonstrated that not only the complete 4-phage cocktail but also each single phage component had anti-inflammatory effects on zebrafish embryos. Moreover, we observed that this effect depends on proteinaceous virion component, but not on phage DNA. In the second part of our work, we investigated on the possible mechanisms through which phages modulate the inflammation in zebrafish embryos. We observed lack of immunomodulatory effects in Myd88-deficient embryos, indicating the involvement of the TOLL-like receptor pathway. In CF embryos, local phage cocktail administration altered neutrophils migration toward an inflammation site, by reducing the chemotactic stimuli. Currently, we are studying the action of the phage cocktail on the human CuFi-1 F508del cell line (characterized by a high basal proinflammatory state), and in primary bronchial epithelial cells, wild-type and F508del, obtained from the "Servizio Colture Primarie" of the Italian Cystic Fibrosis Research Foundation. As a read-out of the action of phages as immunomodulators, we will assess the expression of pro- and anti-inflammatory markers by means of qPCR and ELISA techniques.
The research of new anti-inflammatory agents in a cheap and easy-of-use CF zebrafish model, together with the studies on the effects of phages on CF human cells could speed-up the translational potential of this research.

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Dissecting the transcriptional profile of PDPN^{bright} ECFCs by single cell RNAseq to investigate their role in lymphangiogenesis

Endothelial colony-forming cells (ECFCs) are truly endothelial progenitor cells. In previous studies, we demonstrated that ECFCs express lymphatic markers at variable levels, with a high variability in podoplanin (PDPN) expression that allows the identification of PDPNbright and PDPNdim ECFCs. In order to investigate whether PDPNbright ECFCs may be progenitors of lymphatic endothelial cells (LECs) whose identity is still elusive, in this study we analyzed the transcriptional profile of one PDPNbright and one PDPNdim ECFC colony obtained from healthy donors, by performing a single cell-RNA sequencing (scRNAseq). scRNAseq was performed by using the 10x Genomics Chromium Single-cell system and the Single Cell 3' library preparation protocol; samples were then sequenced on an Illumina NextSeq 500 sequencer. Gene expression data were processed by using an analysis pipeline developed by our Collaborator Dr. Piazza. On the basis of their transcriptional profile, PDPNbright and PDPNdim ECFC colonies were divided into 5 and 6 clusters respectively, with one cluster observed only in PDPNdim ECFC colony. By performing SingleR analysis that allows unbiased cell type recognition, we observed that PDPNdim ECFC colony was composed only by cells characterized with a blood endothelial cell (BEC) phenotype, whereas PDPNbright ECFC colony contained cells endowed with a LEC phenotype, with the exception of one single cluster that contained cells endowed with a BEC phenotype. By using a trajectory analysis, we further allocated cells to branches, and ordered them based on pseudotimes within each branch. Within PDPNbright ECFCs, with this analysis we identified a major branch containing all the cells with LEC phenotype. Detailed analysis of diverging and transition genes are in progress in order to investigate whether ECFCs with LEC phenotype may derive from ECFCs with BEC phenotype as occurring during embryogenesis, and to possibly identify the molecular pathway(s) that promote lymphatic differentiation. In addition, we are performing a NicheNet analysis aimed at dissecting the cell–cell communication networks. In particular, NicheNet predicts ligand–target links between interacting cells by combining their expression data with prior knowledge on signaling and gene regulatory networks. In our setting, by using Nichenet we are looking for the identification of i) factors released by early PDPNbright cells able to promote LEC differentiation, or ii) factors released by the cluster observed only in PDPNdim cells that may either sustain BEC differentiation, or inhibit LEC differentiation.

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Flow cytometric characterization and transcriptomic profiling of dendritic cell subsets in high-grade glioma patients

Background High grade gliomas (HGGs, III and IV grade) are aggressive brain tumors with poor prognosis with a particular tumor ability of suppressing the immune system. Because of the inadequate immune response, the interest in new immunotherapeutic approaches, boosting in particular innate immunity, is growing. Indeed, immunotherapy strategies based on the administration of dendritic cells (DCs) represent a promise in the treatment of HGGs, which are the human cancer that showed the highest susceptibility to DC-based vaccines. DCs are a heterogeneous cell population, composed of different subsets that differ in origin, immunophenotype and function. Because of their unique ability to initiate antitumor immune responses, DCs play a crucial role in cancer immunosurveillance. However, our knowledge on the mechanisms underlying the efficacy of DC-vaccines, as well as the effects of glioma microenvironment on DCs, the subsets of DCs specifically recruited to the tumor site and the impact of glioma on the activatory/tolerogenic profile of DCs is extremely poor. A detailed characterization of tumor-infiltrating DCs is now possible based on the recent molecular definition of distinct DC subsets characterized by transcriptional and functional specialization, together with the availability of new technologies, i.e. multicolor flow cytometry and single-cell RNA-sequencing (scRNAseq) that enable complex multiparameter analysis.
Methods In this study, we enrolled 26 patients affected by HGGs (III and IV grade), followed at the unit of Oncologic Neurosurgery at Humanitas Cancer Center, and 12 healthy donors (HDs). Flow-cytometric characterization of circulating and infiltrating DC subsets was performed on blood from 24 HGG patients and 12 HDs and on 10 tumoral and 2 healthy cerebral tissues, by applying an 18-color panel recently optimized in our lab that allows the identification of distinct DC subsets and their activatory/inhibitory phenotype. scRNAseq was performed on 7 tumoral samples and 2 healthy cerebral tissues of HGG patients, by using 10x Genomics technology. Ingenuity Pathway Analysis (IPA) software was used to investigate the pathways differentially activated or inhibited in DCs.
Results The frequency of circulating pDCs, cDC1s and cDC2s was significantly lower in HGG patients than healthy donors. The reduction of all circulating DC subsets was evident only in patients affected by IV IDH WT gliomas that are the most severe HGGs. Patients undergoing steroid treatment (dexamethasone), needed to reduce cerebral oedema, showed a significant reduction in circulating DCs, independent from glioma grade. The analysis of tissue DCs, revealed that DC subsets did not infiltrate healthy brain parenchyma, but were observed only in HGG tumor tissue. Also tumor-infiltrating DCs were markedly reduced in steroid-receiving patients. By performing scRNAseq, we confirmed the presence of DCs in tumor samples, whereas DCs were mostly absent in healthy brain parenchyma. HGG-infiltrating DCs were distributed into two sub-clusters. By IPA analysis, we observed a functional dichotomy between these clusters, with the smallest being characterized by a general activation and the up-regulation of pro-inflammatory pathways, compared with the largest cell cluster that was characterized by a more inactive phenotype.
Conclusions All DC-lineage DC subsets infiltrate the HGG microenvironment, and their number is impaired by steroid treatment. HGG-infiltarting DCs are characterized by a predominant population of DCs endowed with less active phenotype, but further analyses are in progress, in order to better understand how HGG microenvironment may subvert DC functions.

Erika Di Biase, Giulia Lunghi, Maria Fazzari, Pamela Fato, Simona Prioni, Nicoletta Loberto, Massimo Aureli, Laura Mauri, Sandro Sonnino, Elena Chiricozzi

Attention everyone: it's time for GM1-oligosaccharide

The saccharide chains of the glycoconjugates give the name to the lipids they belong to and, more importantly, often determine their functional role. The specific sequence and conformation of the carbohydrate portion of the monosialylated glycosphingolipid GM1 had long been known to "ganglioside-centric" researchers. But how the glycan came into play to define the neurotrophic and protective role of GM1 was still poor clarified. By change, starting on 2015, a series of multidisciplinary studies and approaches employing the oligosaccharide component of GM1 (OligoGM1), isolated from the parental compound, allowed to shed new light on the mechanisms underlying the properties of GM1 and to recognize a new candidate molecule for the treatment of neurodegenerative diseases. Thanks to the COVID19 period, we have concretized our recent studies on the cascade of events modulated by OligoGM1 as the bioactive portion of GM1, to support neuronal differentiation and trophism together with preclinical studies on the potential of this new molecule to modify the progression of Parkinson's disease.

A true story by
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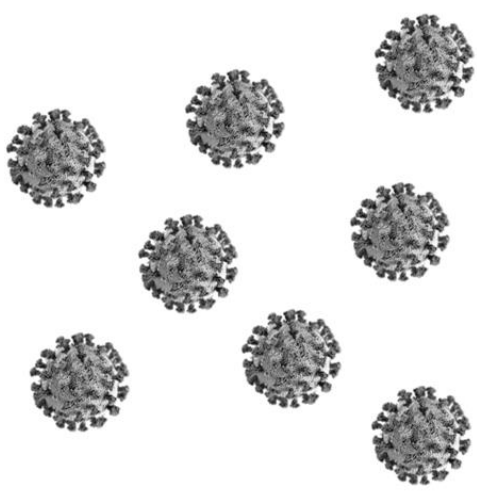
THE SAD STORY OF AN ENZYMATIC REACTION IN THE FRIDGE

"Once upon a time there was a paper to submit and the last two enzymatic reactions whose course was indispensable to finish the experimental work. Everything was going well, but the enemy was lurking. Its name was COVID-19....".

This is the incipit of our story, a story in which two enzymatic reactions were put in the fridge the 9th of March for, we hoped, few days. Instead, two months have passed due to COVID-19 pandemic. However, the time spent at home was not wasted: we prepared most of the manuscript and, reading books and articles, we planned supplementary experimental work to do on our return in the laboratory.

If you would like to know how the story ended
and
the wonderful world of enzymes and how they can be useful tools in organic chemistry....

.... we are waiting for you on September 28th, 2020



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PLAYING EVOLUTION: A GAME OF MOLECULAR ECOLOGY

We have developed a variant of SELEX to explore the emergence and evolution of molecular species under selective pressure. We started with a pool of random-sequence DNA 50mers which underwent recursive steps of affinity capture, amplification and sequencing. Capture was enacted by using magnetic beads coated with DNA 20mers of identical sequence with neither self nor mutual hybridization. 50mers sticking to the beads via hybridization to the capture 20mers were retrieved, amplified and sequenced, to be later exposed to a new affinity capture with equal beads. Each of these cycles is a "generation" in this evolution process.
We find that after 12 generations, out of the immense variety of distinct initial 50mer sequences (of the order of 1015), the population is mainly formed by 11 dominant sequences, or "species".
Because of the pandemic lockdown, we couldn't run more generations, nor restart the experiment from scratch as planned. We had thus more time to better analyze the question: what is making these 11 sequences dominant?
We found that selection provided by this evolution is based not only on the hybridization of the 50mers to the 20mers, but also on the interactions between 50mers, which may compete or cooperate, leading to these major "types" of winning species:

1. The selfish climbers: sequences that attach to the capture 20mers protecting themselves from the interactions of other 50mers by hairpin structures;
2. The promiscuous host and its parasites: sequences that hybridize to the capture 20mers and provide secondary capture sites for other species that don't interact with the beads. Their interactions might shield the host from other interactions competing with its capture.
3. The lucky youngsters: sequences that do not show advantage in the first generations when a large plurality of species is present but start to dominate in the later stages when the competition has simplified.

All this needs to be confirmed with new generations and new independent evolution processes.
Our aim is to provide a simple experimental model enabling quantitative and programmable investigation of topics of general relevance such as the mechanisms of speciation, the coexistence of species in ecosystems or the effects of environmental changes

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Three is better than 2, but is 3 really essential? Few considerations about the need to move to three dimensional analyses when studying the ultrastructure of a synapse

Three dimensional (3D) reconstruction from electron microscopy (EM) datasets is a widely used tool that has improved our knowledge of synapse ultrastructure and organization in the brain. Rearrangements of synapse structure following maturation and in synaptic plasticity have been broadly described and, in many cases, the defective architecture of the synapse has been associated to functional impairments. It is therefore important, when studying brain connectivity, to map these rearrangements with the highest accuracy possible, taking into account the affordability of the different EM approaches to provide solid and reliable data about the structure of such a small complex. The aim of this work is to compare quantitative data from two dimensional (2D) and 3D EM of mouse hippocampal CA1 (apical dendrites), to define whether the results from the two approaches are consistent. We examined asymmetric excitatory synapses focusing on post synaptic density and dendritic spine area and volume as well as spine density, and we compared the results obtained with the two methods. The consistency between 2D and 3D results questions the need -for many applications- of using volumetric datasets (costly and time consuming in terms of both acquisition and analysis), with respect to the more accessible measurements from 2D EM projections. Certainly, 3D EM alone allows the definition of the shape of spines and thus the possibility to sort them according to the canonical shape-based classification through optical microscopy.

Maria Grazia Cattaneo, Raffaella Molteni, Cristiana Perrotta, Clara De Palma

Faraway, so close: 4 ladies, and the way to make the best of a bad time

"Ladies, what do you think about the idea of joining our fields and sharing our knowledge to write a review focused on autophagy?" This is the way how I began my email for Maria Grazia, Raffaella and Cristiana (my former colleague at Sacco Hospital) to propose them a remote collaboration in one of the stranger times of our lives.

I was excited to start my RTD-B at Biometra, but after less than one month, the COVID-19 shut down the whole country. For the first time in our life, we were forced to stay at home. But this did not prevent the wish to face a new challenge with willing girls. We immediately identified autophagy as a cross-cutting topic, and we started writing an abstract that it has been accepted for a special issue on Frontiers in Cell and Developmental Biology. Well done, ladies! Now, we are preparing the manuscript that will be submitted within the end of July.

As well as autophagy is essential for metabolic plasticity and the maintenance of tissue homeostasis, this review has been equally important for us to keep focus on our research, although far from the lab's bench. I am sure that this remote cooperation will be a precious opportunity to deepen our knowledge and to open the way for future partnerships. Of note, we are 4 "girls", thus we have selected "ladies' power" as the nickname of our project. Because we need ladies in science, and science needs ladies.

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Acute and chronic Immune mediated neuropathies during COVID-19: associations and problems

The outbreak of the coronavirus disease (COVID-19) caused by Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2), is rapidly spreading worldwide. More than 7.500.000 confirmed cases worldwide are reported by the SARS-CoV2, with more than 420.000 COVID-19-related deaths by June 15th, 2020.

Typical clinical manifestations of COVID-19 include fever and respiratory symptoms, ranging from mild to severe, and more than one-third of patients experience various neurological symptoms entailing the central (CNS) and the peripheral nervous system (PNS). There are so far almost 30 reported patients with an acute immune-mediated neuropathy (i.e. Guillain-Barré syndrome) in patients with SARS-CoV-2 infection raising the possibility that the virus can directly or indirectly affect the PNS [1]. At the same time, important concerns had been raised for patients with chronic immune-mediated neuropathies on immunosuppressive or immunomodulatory therapies considering the possible higher risk of contracting the infection and/or of experiencing severe manifestations of COVID-19.

Under these premises we developed with the Italian Association of the Peripheral Nervous System (ASNP), the Italian Society of Clinical Neurophysiology (SINC) and the Italian Society of Neurology (SIN) a document to provide clarity and guidance on how operationally manage patients with immune-mediated neuropathies, highlighting the potential rearrangements of care and resetting of clinical priorities [2]. These practical recommendations need however to be individualized according to the severity and the progression of the neuropathy, the local healthcare strategic planning and COVID-19 infection risk.

1. De Sanctis P, Doneddu PE, Vigano' L, Selmi C, Nobile-Orazio E. Guillain-Barré Syndrome associated with SARS-CoV-2 infection: a systematic review. European Journal of Neurology (under revision)

2. Dubbioso R, Nobile-Orazio E, Manganelli F, Santoro L, Briani C, Cocito D, Tedeschi G, Di Lazzaro V, Fabrizi GM. Dealing with immune-mediated neuropathies during Covid-19 Outbreak. Practical Recommendations from the Task force of the Italian Society of Neurology (SIN), the Italian Society of Clinical Neurophysiology (SINC) and the Italian Peripheral Nervous System Association (ASNP). Neurological Sciences 2020; 41: 1345-1348

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Evolution increases LSD1 tuneability: A new alternative splicing isoform

Everyday life stress is a critical risk factor towards the onset of depression and mood disorders. The epigenetic enzyme Lysine Specific Demethylase 1 (LSD1), along with its neuron-specific alternative splicing variant, neuroLSD1, represent remarkable transducers of environmental stimuli in the mammalian brain. LSD1 is a negative modulator of stress-plasticity and memory formation acting as corepressor of the transcription factor Serum Response Factor (SRF). On the contrary neuroLSD1, behaving as dominant negative LSD1 isoform, promotes stimuli-dependent transcription of SRF plasticity-related targets. LSD1 and neuroLSD1 relative ratio is directly modified by stressful events with homeostatic implications tuning down SRF targets transcription and finely contributing to environmental adaptation. Accordingly, an aberrant LSD1 splicing regulation may be responsible for maladaptive forms of plasticity associated with the onset of anxiety-related pathologies, such as depression.

We discovered a further LSD1 primate-specific cryptic exon with an in frame stop codon, exon E8b. We demonstrated that E8b alternative splicing is modulated by a neurospecific splicing regulator recently related to one of the few genetic loci associated with major depressive disorder (MDD). We observed that E8b inclusion into mature transcripts causes a reduction in LSD1 transcript and protein levels through nonsense-mediated mRNA decay (NMD). Moreover, thanks to its differential inclusion within LSD1 isoforms, E8b alternative splicing is able to modify LSD1/neuroLSD1 ratio.

In conclusion, E8b alternative splicing provides LSD1 with an additional modulatory layer contributing to increase complexity of cognitive and social abilities that distinguishes higher primates from other mammals. However, it may also represent a vulnerability hot spot for stress-related neuropsychiatric disorders.

Keywords: LSD1, alternative splicing, NMD, evolution, depression

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In vivo magnetic resonance spectroscopy in the brain of Cdkl5 null mice reveals a metabolic profile indicative of mitochondrial dysfunctions

Mutations in the X-linked CDKL5 gene cause CDKL5 deficiency disorder (CDD), a severe neurodevelopmental condition mainly characterized by infantile epileptic encephalopathy, intellectual disability and autistic features. The molecular mechanisms underlying the clinical symptoms remain largely unknown and the identification of reliable biomarkers in animal models will certainly contribute to increase our comprehension of CDD as well as to assess the efficacy of therapeutic strategies. Here, we used different Magnetic Resonance (MR) methods to disclose structural, functional or metabolic signatures of Cdkl5 deficiency in the brain of adult mice. We found that loss of Cdkl5 does not cause cerebral atrophy but affects distinct brain areas, particularly the hippocampus. By in vivo proton-MR spectroscopy (MRS), we revealed in the Cdkl5 null brain a metabolic dysregulation indicative of mitochondrial dysfunctions. Accordingly, we unveiled defects in the expression of mitochondrial-related genes, while the number and ultrastructure of mitochondria appeared preserved. In conclusion, by using in vivo MRS, we identified novel cerebral metabolic biomarkers for CDD that can be used to foster our comprehension of the neurochemical alterations caused by Cdkl5 deficiency and that highlight the interest of targeting mitochondria as therapeutic strategy for CDD.

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Forgetting COVID-19, how mechanisms of memory inhibition help neutralizing traumatic stressful event

Along with neuronal mechanisms devoted to memory consolidation –including long term potentiation of synaptic strength as prominent electrophysiological correlate, and inherent dendritic spines stabilization as structural counterpart– negative control of memory formation and synaptic plasticity has been described at the molecular and behavioral level. During the COVID-19 pandemic we worked on a paper in which we describe a role of epigenetic corepressor Lysine specific demethylase 1 (LSD1) as a negative neuroplastic factor whose stress-enhanced activity may participate to cope with adverse experiences. Constitutively increasing LSD1 activity via knocking out its dominant negative splicing isoform neuroLSD1 (neuroLSD1KO mice), we observed extensive structural, functional and behavioral signs of excitatory decay, including disrupted memory consolidation. A similar LSD1 increase, obtained with acute antisense oligonucleotide-mediated neuroLSD1 splicing knock down in primary neuronal cultures, dampens spontaneous glutamatergic transmission, reducing mEPSCs.
Remarkably, we observed that LSD1 physiological increase occurs in response to psychosocial stress-induced glutamate signaling. Since this mechanism entails neuroLSD1 splicing downregulation, we conclude that LSD1/neuroLSD1 ratio modulation in the hippocampus is instrumental to a negative homeostatic feedback, restraining glutamatergic neuroplasticity in response to glutamate. The active process of forgetting provides memories with salience. With our work, we propose that softening memory traces of adversities could entail a further stress resiliency-related process in which LSD1/neuroLSD1 ratio modulation may help preserving healthy emotional references via limiting, in a transient time-window, memorization of traumatic experiences.

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"STAY HOME STAY FIT"

During lockdown planned activities related to the project of promotion of correct lifestyles for students of University of Milan and Polytechnic [within the activities of Sustainable Campus] could not be continued.
What could we do?
The idea was to suggest, by way of the website Unimi and Social media, a Series of activities with the aim of improving lifestyle in the lockdown Period.
In fact the COVID-19 emergency and the need of limiting as much as possible the likelihood of infection required from everybody a very big effort. In particular the necessity to stay home continuing, from home, to study and work.
Remaining at home could favor a worsening of lifestyle: "forced" sedentariness also for all those who were used to exercise, different handling of time, possibility of eating at all hours, stress, fear and nervousness that all could potentially invite cigarettes or other substances with the aim of relax.
At the base of our project we utilized a different concept; actually stay home could become a precious opportunity to do exactly the opposite: improve lifestyle.
There is more time for exercising (obviously at home), there is a possibility of preparing food in a healthy way, there is the possibility of better organizing rest and work and several other options for a better use of time.

Goal of the present project was to give simple and concrete indications in order to take lockdown as an opportunity to improve lifestyle fostering health and productivity.
We had very few days to move from an idea to implementation.
We had to contact Governance in order obtain permission to proceed, the office of Public Relations all of the University to get the indispensable collaboration, find other Professors expert in important areas for the project in order to prepare written texts or movies to publish on Social; this is been very intensive work particularly to create video clips without professional help. Everything was organized and prepared as amateurish. It was tiring but also funny..... All those people who were involved we're capable to obtain in a few days a good product: Texts explaining how to be active at home, which is correct posture in order to avoid dorsal pain or headache, how to eat healthy and tasty without putting on weight, how to go to the grocery, how to handle and prevent negative psychological and stress conditions.

The result in the end was gratifying! the initiative called "stay home stay fit" was published on various social websites with the support of the public relations office of the University. The activity was indicated to the RUS (Rete Università per Sviluppo sostenibile) as activity promoted by our University in the Covid Pandemic. Moreover this activity will serve as a basis to continue to offer, within the original project for the promotion of health of the university students, online services independent from emergence of COVID-19 pandemic

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Macrophage morphology correlates with single-cell diversity and prognosis in colorectal liver metastasis

This is the story of a paper that was under revision at the beginning of this "COVID year". We were almost done, only one experiment missing. And then, we were suddenly locked-down. We had to rapidly face the situation, visualize an alternative way to answer the Reviewer's questions, without performing experiments. We reinvented ourselves and went for it. We liked what came out of this reshaping and the Reviewer liked the revised paper. This is our COVID paper, this is what we want to keep of the COVID year.

It has long been known that in vitro polarized macrophages differ in morphology. Stemming from a conventional immunohistology observation, we set out to test the hypothesis that morphology of tumor-associated macrophages (TAMs) in colorectal liver metastasis (CLM) represents a correlate of functional diversity with prognostic significance. Density and morphological metrics of TAMs were measured and correlated with clinico-pathological variables. While density of TAMs did not correlate with survival of CLM patients, the cell area identified small (S-TAM) and large (L-TAM) macrophages that associated with a 5-year disease-free survival rate of 27.8% and 0.2% respectively (P<0.0001). RNA sequencing of morphologically distinct macrophages identified LXR/RXR as the most enriched pathway in large macrophages, with upregulation of genes involved in cholesterol metabolism, scavenger receptors, MERTK and Complement. In single-cell analysis of mononuclear phagocytes from CLM tissues, S-TAM and L-TAM signatures were differentially enriched in individual clusters. These results suggest that morphometric characterization of TAMs in human colorectal liver metastases captures individual populations, corresponding to single-cell clusters and associated with distinct clinical outcome and transcriptional profiles. Quantitative analysis of macrophage morphology can serve as a simple readout of functional diversity with prognostic significance.

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Conformational selection mechanisms in human prion protein mutants bound to a camelid antibody fragment

The conformational selection paradigm plays a central role in pharmacological approaches targeting highly dynamic protein conformations where potential interacting partners are able to stabilize a particular conformation. Proteins displaying a continuum of folding states are involved in cellular signalling and regulation, but also in pathological amyloidal aggregation processes associated with neurodegenerative diseases. Immunotherapies using antibodies to target the aggregation of flexible proteins are a highly perused approach. Prions, the causal agents of transmissible spongiform encephalopathies, have a protein only self-propagating infectious mechanism that has represented a prominent target for immunotherapies during the last decades. Here, we perform an extensive structural analysis of the human prion protein (HuPrP) bound to a camelid antibody fragment denoted as nanobody (Nb). We used the Nb to solve the X-ray diffraction (XRD) structures of two HuPrP carrying the V210I and E219K mutations linked to genetic human prion diseases. Notably, the XRD structures of the mutants show identical 3D architectures as the wild-type HuPrP when bound to the Nb. We addressed this issue through molecular dynamics simulations of the free proteins and their comparison with nuclear magnetic resonance spectroscopy (NMR) data from previous investigations on HuPrP in solution. We show that the unbound mutants populate different folding conformations that are very similar to the ones identified through NMR but rarely converge into the Nb-bound states. In particular, the E219K conformational space includes less populated conformations that are very close to the ones that are bound to Nb, whereas the V210I dynamic behaviour does not display significant similarity with the structure bound to Nb. These aspects of HuPrP dynamics supports the conformational selection model of molecular recognition for the former mutant and the induced fit model for the latter. Moreover, they underline the efficacy of this Nb to stabilize the folding of aggregation prone HuPrP mutants responsible for genetic human prion diseases.

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Hedgehog and HDAC6 inhibition in the treatment of chemoresistant liquid and solid tumors

Aberrant activation of the Hedgehog (Hh) signaling pathway is responsible for the chemoresistance of different forms of cancer including glioblastoma (GBM) and acute myeloid leukemia (AML). Several small molecule inhibitors targeting this pathway have been developed, such as cyclopamine. However, mainly half of the patients with Hh-dependent tumors show primary or secondary resistance to standard chemotherapy due to unknown mechanisms. In recent years, it has been discovered that cancer cells fail to present the primary cilium (PC), an organelle that in mammals is involved in the Hh signalling transduction. One of the key players in PC stabilization is the histone deacetylase HDAC6 as deacetylating alpha-tubulin promotes microtubule disassembly and cilium reabsorption. Interestingly, HDAC6 is overexpressed in several tumours and drugs to selectively prevent HDAC6 activity are currently in use. Therefore, the inhibition of HDAC6 might be a promising therapy for cancer cells. To dissect the role of Hh signaling and HDAC6 dysregulation in the development of chemoresistance, we firstly performed in silico analyses. By means of GEPIA2 tool we identified a positive correlation between HDAC6 and Hh genes and between HDAC6/Hh genes and multi drug resistance genes (MDR) in GBM and AML datasets. Therefore, we generated a zebrafish model with Hh hyperactivation through the injection of human and zebrafish Hh mRNA. By means of qPCR techniques, we confirmed the positive correlation between Hh, hdac6 and MDR genes. For the in vivo characterisation, we overexpressed Hh in the Tg(CD41:GFP) zebrafish line which expresses the GFP in the Hematopoietic Stem Cells (HSCs) population. Hh overexpression determined a pre-leukemic phenotype with an augmented number of HSC and increased expression of MDR. To rescue the hematopoietic phenotype and MDR expression, we compared the effect of Cyclopamine and TubastatinA (TubA) treatments, two specific inhibitors of Hh and HDAC6 respectively. Both compounds reduced the MDR expression but only TubA treatment rescued the number of HSC. Moreover, preliminary experiments using U87MG as cell line model of GBM that express both Hh and HDAC6, demonstrated that TubA treatment efficiently reduces cell viability and migration. These results suggest that HDAC6 inhibitors, such as TubA, could be a possible alternative to Hh inhibitors for single or combination therapy with standard chemotherapeutic treatments in chemoresistant tumours such as AML and GBM.

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TRANSCRIPTIONAL PROFILING ANALYSIS AND FUNCTIONAL PREDICTION OF MS4A4A IN HUMAN MONOCYTES

Membrane-Spanning 4-domain subfamily A (MS4A) proteins are emerging as a family of proteins involved in the formation of lipid raft-associated signaling complexes. Our group reported the expression of MS4A4A in Tumor-Associated Macrophages (TAM), both in humans and in mice tissues. MS4A4A associates with itself and with two other members of MS4A family (MS4A6A and MS4A7), and localizes in lipid rafts, where it interacts with the pattern recognition beta-glucan receptor Dectin-1 upon its engagement by zymosan. Gene-targeted animals demonstrated that MS4A4A supported Dectin-1-mediated protection against metastases for glucan-positive tumors.

TAMs are a highly heterogeneous cell population with regard to their activation, function, and spatial distribution within tissues with a key role on local tumor progression and distant spreading, with well defined prognostic implications. Despite the wide range of studies about TAMs, their origin is still debated. At this stage, it is reasonable to assume that their presence in the tumor site represents a mixture of accumulation and possibly local replication of both tissue-resident and monocyte-derived macrophages. Based on this, we explored weather MS4A4A is also expressed by monocytes and found that the protein is selectively expressed by a fraction of the non-conventional CD16pos monocyte subset. To get insights on the profile of MS4A4A+ monocyte subpopulation, we isolated and sequenced CD16neg/MS4A4Aneg, CD16pos/MS4A4Apos and CD16pos/MS4A4Aneg monocytes. The transcriptomic analysis revealed 1,397 differentially expressed genes within the possible comparisons between two subsets. MS4A4A has been confirmed enriched in the subset of non- classical monocytes as well as MS4A7, while MS4A6A transcript was upregulated in the CD14pos classical monocytes. Different bioinformatics tools were applied, including Ingenuity pathway analysis (IPA) software and Gene Set Enrichment Analysis (GSEA) analysis, and we were able to found a clear enrichment of the FcγR and FcεR pathways in MS4A4Apos subset of CD16pos monocytes.

Given that Fc receptors expressed by monocytes/macrophages are exploited in cancer immunotherapy to elicit antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC) of cancer cells, the role of MS4A4A in antibody-dependent FcR- mediated anticancer activity is now being evaluated.

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Digenic inheritance of subclinical variants in Noonan Syndrome patients: an alternative pathogenic model?

During COVID period I was sick and probably I had COVID-19. Fortunately, I was affected by the mild form, but in that period none could foresee the evolution of the infection. I was isolated with my elderly parents and I do not deny that I was got caught several times in discouragement, seen perspectives of cure = 0 in case we needed it. Friends and some colleagues encouraged me with their calls that I will never forget because they helped me to get through three weeks physically and psychologically really hard and 50 days of isolation. In this context, I found the strength to prepare the lessons of the Genetics course and to discuss and finish with collaborators the drafting of the work that I present to you and which has recently been accepted for publication.

Noonan syndrome (NS) is an autosomal-dominant disorder with variable expressivity and locus heterogeneity. Despite several RAS pathway genes were implicated in NS, 20-30% of patients remain without molecular diagnosis, suggesting the involvement of further genes or multiple mechanisms. Eight patients out of 60, negative for conventional NS mutation analysis, with heterogeneous NS phenotype were investigated by means of target resequencing of 26 RAS/MAPK pathway genes. A trio was further characterized by means of whole exome sequencing. Protein modelling and in silico prediction of protein stability allowed to identify possible pathogenic RAS pathway variants in four NS patients. A new c.355T>C variant in LZTR1 was found in patient 43. Two patients co-inherited variants in LRP1 and LZTR1 (patient 53), or LRP1 and SOS1 genes (patient 67). The forth patient (56) carried a compound heterozygote of RASAL3 gene variants and also an A2ML1 variant. While these subclinical variants are singularly present in healthy parents, they co-segregate in patients, suggesting their addictive effect and supporting a digenic inheritance, as alternative model to a more common monogenic transmission. The ERK1/2 and SAPK/JNK activation state, assessed on immortalized lymphocytes from patients 53 and 67 showed highest phosphorylation levels compared to their asymptomatic parents. These findings together with the lack of their co-occurrence in the 1000Genomes database strengthen the hypothesis of digenic inheritance in a subset of NS patients. This study suggests caution in the exclusion of subclinical variants that might play a pathogenic role providing new insights for alternative hereditary mechanisms.

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Unveiling the therapeutic potential of HDAC8 inhibition

The use of pan-histone deacetylase inhibitors (HDACi) for disease treatment has gained an interest in recent years, albeit exhibiting low specificity, variable efficacy and side effects. Therefore, the identification of the potential of individual HDACs as pharmacological targets may improve therapy outcome, circumventing the adverse effects of pan-HDACi. Histone deacetylase 8 (HDAC8) is a class I HDAC that possesses a unique structure among HDACs, which has allowed the development of highly specific inhibitors, such as PCI-34051. By using both cell lines and zebrafish (Danio rerio), we assessed the feasibility of PCI administration as a pharmacological approach in the treatment of two pathologies characterized by HDAC8 overexpression: acute myeloid leukaemia (AML) and Duchenne muscular dystrophy (DMD). Concerning AML, we demonstrated that PCI treatment rescued hematopoietic stem cell (HSC) expansion caused by hdac8 overexpression in zebrafish embryos, by inducing cell cycle arrest and apoptosis. Our analysis identified both p53-dependent apoptosis and canonical WNT pathway modulation as mechanisms underlying the anti-leukaemic effect of HDAC8 inhibition by PCI. Also, in DMD myotubes and in dystrophic zebrafish embryos, PCI administration ameliorated the skeletal muscle differentiation. To gain insight in HDAC8 mechanism of action, we performed acetylome profiling in zebrafish embryos treated or not with PCI and identified cytoskeleton proteins as HDAC8 targets, including α-tubulin. Indeed, we observed that α-tubulin acetylation was increased in dystrophic zebrafish with high hdac8 expression and that PCI treatment not only restored normal acetylation but also rescued microtubule architecture alterations, which are a typical DMD feature. Our results suggest that selective inhibition of HDAC8 by PCI-34051 possesses a promising potential both as a therapeutic approach for DMD treatment and as an anti-leukaemic drug.

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Simulating competitive RNA/DNA hybrid – Immune complex hybridization in a non-simulated quarantine

MiRNAs are widely recognized as reliable biomarkers for various diseases. Due to the low abundance the miRNAs usually come in, detection and quantification often requires time consuming and complex protocols. We utilized our novel, label-free, optical biosensor platform, Reflective Phantom Interface (RPI), and developed a rapid (<1.5h) and sensitive (5 pM) miRNA detection assay. We based this assay on two step amplification using surface tethered DNA probes that bind the miRNA, forming a DNA/RNA hybrid and targeted these hybrids with RNA/DNA specific antibody (Ab1) and polyclonal secondary antibody (Ab2). Using this two step amplification procedure we achieved a 100 fold signal amplification. Because RPI allows the detection of various analytes, without any washing steps, this assay allowed us to study the formation of immune complexes on surface under this competitive regime, since the antibodies can interact also in the bulk solution, yielding lower available concentration of Ab1 following the injection of Ab2. During this quarantine I used the time tied to my computer to perform simulations based on simple numerical model in order to understand the behaviour of the system and optimize the assay perfomance. Results obtained from this simulations suggest a strong influence of solution formed Ab1-Ab2 pairs, and resulting lower effective concentration of Abs, on the complete binding curve. Furthemore, this results allowed us to further optimize our assay and achieve a >100 fold amplification. This results played a big role in the development of the assay and we are hoping to soon submit a paper based on this assay.

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Kicking and breaking at the microscale: investigation of the yielding of soft materials with an optofluidic micro-rheometer

The relevance of soft materials for our daily life is nested in their multiple length and time scales, rich mechanical behaviour, viscoelastic response and flow properties. In particular, yield stress materials (like mucus, ketchup or hair gel) deform as elastic solids or flow as viscous liquids, depending on the applied stress. Correspondingly, such materials can stably trap particles if their size and density do not exceed threshold values. The origin and conditions of this mechanical solid-to-fluid transition display several open issues, related e.g. to the stress distribution within the material and the role of microstructure and dynamics [1]. We can directly elucidate the yielding mechanisms comparing the macroscale mechanical properties with the microscale behaviour, e.g. with tools like optical tweezers, which are however limited in terms of attainable forces and are thus often insufficient to induce local failure.

Here, we demonstrate that a recently developed microrheology setup, based on a microfluidic chip and optical scattering forces acting on a microbead [2], is ideally suited for yield stress measurements, easily reaching hundreds of pN and thus providing access to a wide range of phenomena. The design consists of one or two counter-propagating infrared beams carried into a microchannel by two facing waveguides, realized by femtosecond laser fabrication in a glass substrate, and impinging onto a microbead. The intensity of the beams can be temporally tuned by a shutter, or by an integrated optical modulator oscillating between 0.01 and 30 Hz [3].

We perform microscale creep and recovery experiments on swollen microgels and colloidal fibre networks in a surfactant background [4]. We find qualitative and quantitative agreement with bulk behaviour, from elastic deformation to viscous, shear thinning flow for increasing stresses. However, the trajectories of the microbead proceed by discrete jumps, bearing distinctive trace of the rearrangements within the material. By seeding the samples with small, passive tracers, we characterize the deformation field and estimate the extent of the fluidized region around the microsphere.

Furthermore, we investigate the behaviour of the microbead upon application of oscillatory stresses at different frequencies, which provide a complementary access to yielding conditions. Upon increasing stress (and strain) amplitude, we find transition from linear to nonlinear response, again in quantitative agreement with macroscopy rheology.

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