The One Health 2.0

Beyond the boundaries

TeCSBi PhD Meeting 2023

15th PhD Meeting

September 25-27, 2023

Certosa 1515, Avigliana (TO)

Istituto di Ricerche Farmacologiche Mario Negri, Milano (MI)

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Day 1
Monday 25 September 2023

9.00-9.20	**Opening - Paola Branduardi**	
9.20-9.40	T1	Paolo Pizzul *"The role of Rif2 and Sae2 in MRX complex plasticity during DNA damage response"*
9.40-10.00	T2	Elisa Perciballi *"Exploring the correlation between bioenergetic profile and ALS pathogenesis in fibroblasts of TARDBP p.G376D mutation carriers."*
10.00-10.35 (5 minutes each)	FT1	Stefano Bianchini *"Roles of phytoextracts in colorectal cancer prevention and therapy"*
	FT2	Maddalena Bracchi *"Mimicking extracellular matrix (ECM) features for meniscal regeneration: from biomolecular signatures to biomaterials design"*
	FT3	Chiara Florindi *"Non-genetic optical modulation of cardiac excitable cells by membrane-targeted azobenzene photoswitches"*
	FT4	Francesco Abbiati *"Quercetin extends yeast chronological lifespan, reducing oxidative stress and modulating carbon metabolism"*
	FT5	Miriam Kuku Afanga *"Identification of miRNA-based biomarkers predictive of lung cancer treatment response and mechanisms involved in lung cancer progression"*
	FT6	Alice Italia *"Study on the impact of Toll-like Receptor 4 (TLR4) modulation in rare inflammatory-fibrotic diseases"*
	FT7	Barbara Zerbato *"Exploiting DNA damage protein O-glycosylation to enhance chemotherapy in pancreatic cancer"*
10.35-11.20	**Coffee Break & Poster Session**	

11.20-11.40	T3	Chiara Baioni *"Killing cancer cells by targeting the tumor microenvironment: study of TRAIL subcellular and extracellular localization in a CAFs model"*
11.40-12.00	T4	Michela Galli *"Interplays between nucleases and helicases in the DNA damage response"*
12.00-12.20	T5	Giacomo Ducci *"Integration of omics and functional metabolism data to understand cancer progression rearrangements in bladder cancer advanced in vitro models"*
12.20-12.40	T6	Chiara Frigerio *"Understanding the interconnections between DNA-RNA hybrids and the DNA damage response"*
12.40-13.00	T7	Elisa Dama *"The challenge of lung cancer early diagnosis: development of a circulating miRNAs signature using liquid biopsy"*
13.00-14.30		**Lunch**
14.30-15.20	KL1	**Andrea Camattari** TBA
15.20-15.40	T8	Roberta Pensotti *"Caenorhabditis elegans in aging research: characterization of healthspan parameters during lifespan"*
15.40-16.00	T9	Filippo Testa *"Synthesis and spatio-temporal imaging of nanoparticles in cultured cells by Ultrafast Electron Microscopy for tumor theranostics"*
16.00-16.20	T10	Giulia Tomaino *"Recombinant vault nanoparticle as a potential tool for the targeted delivery of siRNA as therapeutic molecule"*
16.20-16.50		**Coffee Break**

16.50-17.10	T11	Giulia Motta *"Adverse outcome pathways oriented toxicology in in vitro systems for implementing the safety-by-design of new nanomaterials: submerged and Air-Liquid-Interface exposure"*
17.10-17.30	T12	Sara Fumagalli *"SKIOME project: unveiling the skin microbiome from metadata retrieval to metagenomics meta-analysis with an innovative tool and a curated data collection"*
17.30-18.20	KL2	**Francesco De Angelis** TBA

Day 2
Tuesday 26 September 2023

9.00-9.20	T13	Federica Barbugian *"3D-bioprinted hybrid hydrogels simulating the extracellular matrix dynamics in vitro"*
9.20-9.40	T14	Stefania Pagliari *"Development of a Pressurized Liquid Extraction method for the recovery of glucosinolates from Camelina sativa (L.) Crantz seed by-products and evaluation of their potential bioactivities."*
9.40-10.15 (5 minutes each)	FT8	Tommaso Sassi *"Heterologous expression of oxidative enzymes in microbial cells for bioconversion of raw materials to produtcs of interest for the pharmaceutical industry"*
	FT9	Chiara Giustra *"Metabolomics approach coupled with multivariate analysis of different parts of Prosopis cineraria (Ghaf) for evaluation of their biological activity"*
	FT10	Laura Beretta *"NO PAINs NO GAINs: the importance of TR-FRET interference assays for the validation of true-positive hits"*
	FT11	Elena Bernasconi *"Advanced biomaterials engineered for the production of sustainable compounds"*
	FT12	Rosa Ranalli *"The first national assessment of ecosystem pollination service in Italian urban environments: first results and future perspectives"*
	FT13	Elisa Toini *"Phylogenetic analysis for research of plant active compound"*
	FT14	Giulia Ghisleni *"UniBiome project: new microbiome-inspired approaches for a sustainable urban regeneration of Universities"*

10.15-11.00		**Coffee Break & Poster Session**
11.00-11.50	KL3	**Francesca Granata** TBA
11.50-12.10	T15	Alessandro Marchetti *"Role of glycoside hydrolases family 3 in Antarctic marine bacterium"*
12.10-12.30	T16	Beatrice Negrini *"Safe and sustainable nano-enabled antimicrobials to reduce the presence of contaminants of emerging concern in the aquatic environments: a focus on CuO-based nanomaterials"*
12.30-12.50	T17	Pietro Butti *"Natural colors production in engineered microbial cell factories: a synthetic biology challenge"*
12.50-13.10	T18	Emiliano Pioltelli *"From Pollinator Diet to Human Nutrition: the surprising insights from pollination ecology"*
13.10-14.30		**Lunch**
14.30-15.20	KL4	**Gerard Griffionen** TBA
15.20-15.40	T19	Marta Simonetti *"Implementation of a bioeconomy strategy in the textile industry"*
15.40-16.00	T20	Vittorio Senatore *"Yeast fermentation for the upcycling of PET monomers"*
16.00-16.30		**Coffee Break**
16.30-16.50	T21	Giuseppe Silvestri *"Insights into thermodynamic and structural properties of biological systems from high-performance computing-based simulations"*
16.50-17.10	T22	Mirko Zago *"Novel biotechnological route for the production of low molecular weight (LMW) esters"*

17.10-17.30 T23 Thomas Vernay

"Paired omics for the study of antibiotic and biofilm modulating agents in rare actinomycetes"

Day 3
Wednesday 27 September 2023

9.30-9.50		**Opening at Mario Negri - Paolo Bigini**
9.50-10.25 (5 minutes each)	FT15	Serena Seminara *"Contribution of the Wiskott-Aldrich syndrome protein in microglia and brain resident macrophage physiological and pathological functions"*
	FT16	Maria Chiara Barbera *"Reactivation of miR-29 to mitigate tumor adaptation and hormone resistance in prostate cancer"*
	FT17	Alessia Lambiase *"Characterization of plant bioactive molecules in eukaryotic models of Parkinson's disease"*
	FT18	Alessia Metallo *"Intracellular Ca2+ dynamics modulation by istaroxime and its metabolite in pulmonary artery smooth muscle cells"*
	FT19	Angela Maria Giada Giovenale *"The impact of Rai1 haploinsufficiency on lipid metabolism and autophagic flux in Smith-Magenis Syndrome"*
	FT20	Giorgia Ruotolo *"Primary cilium characterization in Joubert Syndrome somatic cells"*
	FT21	Marco Barreca *"Multidimensional data integration to capture intrinsic and extrinsic mechanisms driving treatment benefit in breast cancer"*
10.25-10.45		Sponsor
10.45-11.30		**Coffee Break & Poster Session**
11.30-12.20	KL5	**Alessandro Scardua** TBA
12.20-12.40	T24	Giulia De Simone *"Unveiling dietary habits in elderly: hippuric acid and food-derived markers in frailty"*

12.40-13.00	T25	Marina Meroni *"The combination of PPARg and RXR agonists induces adipocytic differentiation in myxoid liposarcoma"*
13.00-14.30		**Lunch**
14.30-14.50	T26	Serena Petrella *"Identification of new therapeutic strategies for mucinous ovarian carcinoma"*
14.50-15.10	T27	Giulia Dellavedova *"Strategies to overcome PARP inhibitors drawbacks: from biomarkers selection to novel therapies"*
15.10-15.30	T28	Laura Formenti *"Ovarian cancer and PARP inhibitors: the emerging role of mitochondrial metabolism"*
15.30-15.50	T29	Annalisa Morelli *"Lung disorders treatment: Investigating the impact of inhalable nanoformulations in a murine model of pulmonary fibrosis"*
15.50-16.10		Sponsor
16.10-16.45		**Coffee Break**
16.45-17.35	KL6	**Luigi Naldini** TBA
17.35-18.00		**Greetings & Closing Remark**

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Abstract Book

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Istituto di Ricerche Farmacologiche Mario Negri, Milano (MI)

T01

The role of Rif2 in MRX complex function at double-strand breaks

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DNA double-strand breaks (DSBs) are highly cytotoxic lesions that have to be repaired in order to guarantee genomic stability. The MRX/MRN complex (Mre11, Rad50, Xrs2/NBS1) recognises and initiates double-stranded DNA break repair and activates the Tel1/ATM kinase in the DNA damage response. MRX binding and processing activities on DNA are regulated by transitions between different conformations promoted by Sae2/CtIP and Rif2 proteins, and by ATP binding and hydrolysis. Sae2 and Rif2 regulate MRX functions in opposite manners by interacting with Rad50 and influencing ATP-dependent Mre11-Rad50 conformational changes. Rif2 is mainly present at telomeres, where it inhibits MRX-dependent Tel1 activity[1]. Rif2 is also recruited at DSBs[2], but its function has not been fully deciphered yet. To better understand the role of Rif2 at DSBs we design a N-terminal[3] *rif2* mutation that enhances Rif2 inhibitory functions by increasing its interaction with MRX. This mutation leads to a defect in activation of Tel1/ATM, leading to impaired DSB end-tethering. This variant reduces association of Tel1 with DNA DSBs but not of MRX, indicating that Rif2 can negatively regulate Tel1 recruitment independently of the control of MRX binding at DNA ends, suggesting that Rif2-bound Rad50 is not competent for Tel1 binding.

Keywords: double-strand break / MRX / Rad50 / Rif2 / Tel1 / *S. cerevisiae*

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T02

Exploring the correlation between bioenergetic profile and ALS pathogenesis in fibroblasts of TARDBP p.G376D mutation carriers

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Amyotrophic Lateral Sclerosis is an incurable neurodegenerative disease in which the death of motor neurons causes weakness and wasting of muscles until death [1]. About 5-10% of cases are due to genetic mutations inherited from a family member (fALS). ALS patients display heterogeneous phenotypes, and the differences can also be seen among individuals bearing the same mutation.

TDP-43, a versatile RNA/DNA binding protein involved in RNA metabolism, is associated with a risk of the disease [2,3], but the mechanisms by which it exerts the pathogenetic effects are still not well known.

For these reasons, in this work we studied fibroblasts derived from an Italian family bearing the p.G376D mutation in the glycine-rich domain, a critical component of the protein [4]. We performed a metabolic analysis of cells derived from symptomatic and asymptomatic carriers and our data suggest that the G376D mutation causes an imbalance in the oxidative stress/antioxidant defence system which leads (or may be caused) to metabolic alterations. Generally, ALS cells display mitochondria impairments that possibly cause a deregulation of the oxidative phosphorylation/glycolysis balance inducing a switch toward a predominantly glycolytic metabolic profile at late disease stage. Of note, our data suggest that the mitochondria impairments represent an underlying family trait compensated in different ways depending on individual background.

Keywords: ALS, TDP-43, metabolism, mitochondria

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FT01

Roles of phytoextracts in colorectal cancer prevention and therapy

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Colorectal cancer (CRC) is one of the most common types of cancer worldwide[1]. Therefore, many drugs have been developed to treat this disease, although their efficacy is variable and may cause strong side effects. Natural compounds present in plants, such as polyphenols, terpenes, and carotenoids, have shown antiproliferative, antioxidants and anti-inflammatory properties in numerous *in vitro* experiments conducted on cancer cells[2], possibly representing a new therapeutic approach. Hence, the aim of the project is to test the aforementioned properties of phytoextracts derived from leaves of various plant species on different CRC cell lines. Initially, these phytoextracts will be screened in order to find the most promising candidates, that will then undergo further molecular and biochemical characterization to elucidate the mechanisms through which they act.

Keywords: Colorectal cancer, phytoextracts, antiproliferative, antioxidant.

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FT02

Mimicking extracellular matrix (ECM) features for meniscal regeneration: from biomolecular signatures to biomaterials design

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The menisci are crescent-shaped fibrocartilaginous structures found in the knee joint between the femoral condyles and the tibial plateau which primary function is the weight distribution [1]. They are composed of three distinct regions with different cell populations, extracellular matrix (ECM) components and morphological features [2]. However, due to its poor vasculature, poor healing capacity and complex structure, the in vitro regeneration of functional meniscus remains particularly challenging [3].

Here in this work, the ECM composition and the in vitro regeneration of the meniscus were investigated for tissue engineering purposes. In particular, the ECM profile was analysed with different stains and scanning electron microscope (SEM) images obtained from patient-derived tissues to study the differential ECM signatures in tissue damaged by trauma or pathologies. The stress-strain distribution in the tissue was then explored with finite element models (FEM) from MRI images.

Finally, to recreate the ECM composition, two different hydrogels were formulated and characterized with chemical, physical, mechanical, and biological characterizations and compared to the native tissue.

Keywords: meniscus, biomaterials, regeneration, knee, degeneration

References

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FT03

Non-genetic optical modulation of cardiac excitable cells by membrane-targeted azobenzene photoswitches

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The use of light to control the activity of different cell-types is emerging as a promising approach for many applications in cell biology and cardiac research as it offers minimal invasiveness, unprecedented spatio-temporal selectivity, and reversibility compared to more standard stimulation cues [1]. Here, we propose two recently synthetized intramembrane azobenzene photoswitches [2] (Ziapin2 and NO_2–2Pyr) for optical modulation of cardiomyocytes (CMs) electrical properties.

The light-mediated stimulation process has been studied by applying several techniques to detect the effect on CMs. In particular, we used electrophysiological measurements, time-resolution epifluorescence and motion video-analysis to investigate the effect of the molecules on passive cellular properties, membrane potential (Vm), intracellular Ca^{2+} dynamics and cell contractility, respectively.

Both molecules partition into the plasma membrane and lead to Vm modulation. In particular, Ziapin2 mechanical photomodulation causes a transient hyperpolarization followed by a delayed depolarization that triggers action potential generation and contraction. Conversely, NO_2–2Pyr electrical photomodulation generates a sub-threshold depolazation of few mV, strongly light-intensity dependent.

Altogether, these data provide the proof of concept that Ziapin2 and NO_2–2Pyr represent viable tools for the geneless optomodulation of cardiac cell electrical behaviour and open interesting perspectives for their application in cardiac cellular electrophysiology.

Keywords: non-genetic light stimulation, photoactuators, cardiac cellular modulation, cellular electrophysiology.

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FT04

Quercetin extends yeast chronological lifespan, reducing oxidative stress and modulating carbon metabolism

Francesco Abbiati,[1] Stefano Angelo Garagnani,[1] Ivan Orlandi[1] and Marina Vai[1]

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Quercetin (QUER) is a natural polyphenolic compound belonging to flavonols and it is present in the human diet *via* vegetables and fruits[1]. QUER is marketed as a nutraceutical due to its health benefits[2], among which anti-aging ones, but target molecules/pathways underlying its pro-longevity potential have yet to be fully clarified.

We investigated QUER effects on aging process in the context of chronological aging, the established yeast model that simulates cellular aging of postmitotic quiescent mammalian cells[3].

We found that QUER supplementation at the onset of the chronological aging, namely at the diauxic shift, significantly increases the chronological lifespan. Consistent with antioxidant properties of QUER, this extension takes place in concert with a decrease of oxidative stress. In addition, we found that QUER supplementation elicited an enhancement along the glyoxylate/gluconeogenesis axis resulting in an increased utilization of C2-compounds and a higher accumulation of trehalose, disaccharide fundamental for longevity in yeast.

In perspective, since the key enzyme of gluconeogenesis, the phosphoenolpyruvate carboxykinase Pck1, is regulated by Sir2, founding member of Sirtuins, we will analyse in detail the activity of this NAD^+-dependent deacetylase in QUER-supplemented cells.

Keywords: Quercetin, aging, yeast, *Saccharomyces cerevisiae*, oxidative stress, glyoxylate shunt, gluconeogenesis, trehalose.

References

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FT05

Identification of miRNA-based biomarkers predictive of lung cancer treatment response and of mechanisms involved in lung cancer progression

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Non-small-cell lung cancer (NSCLC) is the main cause of cancer-related deaths worldwide1. This is mainly due to the lack of diagnostic/prognostic biomarkers to improve early diagnosis and ameliorate treatment response2. Recently, microRNAs (miRNAs) were reported to contribute to cancer progression and therapy resistance in NSCLC3. Here, we propose to functionally map the whole miRNome involved in NSCLC progression and therapy resistance, by taking advantage of a lentiviral-based library overexpressing the human miRNome (N=2580) in relevant lung cancer experimental models. Our findings will also contribute to the identification of predictive miRNA-based biomarkers and molecular targets to aid the development of alternative therapeutic approaches in unresponsive lung cancer.

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FT06

Impact of Toll-like Receptor 4 (TLR4) chemical modulation by small-molecular antagonists in rare inflammatory-fibrotic diseases

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We present here preliminary data on the possible impact of chemical modulation of TLR4 activity on fibrosis progression. Our labs developed several synthetic molecules that showed biological activity in inhibiting TRL4 activation by LPS in a dose-dependent way. Our in vitro experiments with purified receptors suggested that the antagonistic action is due to the interaction of these compounds with MD-2 and CD14 co-receptors [1].

Fibrosis is an outcome of the repair response to tissue damage caused by inflammation. When the fibrotic process is excessive or dysregulated it leads to a pathological condition that can affect different organs and functions. Here, is now clear that inflammation, which however is not the only trigger, plays a key role in the critical cellular process of fibroblasts activation that leads to fibrosis upset [2].

The recent discovery of a complex crosstalk between fibrosis progression and inflammatory pathways suggests the central role of TRL4 and its potential as new drug target[3].

Thus, the aim is to identify new or old compounds acting on TR4 to block or prevent the fibrosis development exploiting synthetic TRL4 antagonists.

Principal focus is on Idiopathic Pulmonary Fibrosis (IPF) and Morphea which are rare fibrotic pathologies where a pivotal role of TLR4-mediated inflammation has been observed [4 5].

Keywords: TRL4 antagonist, Fibrosis, Inflammation

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FT07

Exploiting DNA damage protein O-glycosylation to enhance chemotherapy in pancreatic cancer

Barbara Zerbato[1], Virginia Brancato[1,2], Chiara Lattuada[1], Francesca Mossi[1], Yang Gang[3], Taiping Zhang[3], Marina La Chimia[4], Domenica Scumaci[4], Ferdinando Chiaradonna[1]

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Pancreatic cancer (PC) is the fourth leading cause of cancer death[1]. Presently, chemotherapy based on gemcitabine (GEM) is one of the most widely used schemes for PC, but this standard approach appears poorly effective in patients due to resistance development[2]. GEM-resistance is related to cancer metabolism rewiring. Noteworthy, PC exhibits an increased flux through the Hexosamine Biosynthetic Pathway (HBP), involved in protein glycosylation. Recently, it has emerged that HBP regulates DNA damage response, activating DNA Damage Repair (DDR) mechanisms[3]. The project aims to delineate if HBP inhibition and protein O-glycosylation impact pancreatic cancer chemotherapeutics resistance. To this end, we decided to use FR054, a specific PGM3 inhibitor, combined with GEM. Our preliminary results show that this combined treatment enhances cell death in several PC cells through the induction of cell cycle arrest and an increase in DNA damage. Interestingly, FR054 co-treatment prevents the GEM-induced intra-S-phase checkpoint activation since a significant decrease of CHK1 and CHK2 phosphorylation is observed. These findings suggest a direct role of protein O-GlcNAc in S-phase checkpoint control and in DDR proteins function, providing a link between cancer -specific metabolic reprogramming and potential therapeutic response.

Keywords: Pancreatic cancer, Hexosamine Biosynthetic Pathway, DNA damage

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T03

Killing cancer cells by targeting the tumor microenvironment: study of TRAIL subcellular and extracellular localization in a CAFs model

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Tumor Necrosis Factor Related Apoptosis-Inducing Ligand (TRAIL) is physiologically expressed as a type 2 transmembrane protein that can be cleaved into a soluble form, less active than the membrane-bound one [1]. TRAIL has received great attention as a potential anticancer agent for its ability to selectively induce apoptosis in cancer cells throw a p53-independent mechanism. This led to the development of TRAIL-receptors agonists which, however, failed to translate into clinics due to poor pharmacokinetics and weak induction of apoptosis [2]. To overcome these shortcomings, TRAIL gene therapies can be developed.

This project aims at giving a proof of concept that, regardless of the gene therapy vector, Cancer Associated Fibroblasts (CAFs) – the most accessible cells at the tumor microenvironment [3] – can be used as a TRAIL depot, that TRAIL localizes into the membrane of TRAIL$^+$ CAFs-released exosomes and that TRAIL$^+$ exosomes can trigger apoptosis in cancer cells. To these aims, we transfected a CAFs model with TRAIL mRNA and we investigated TRAIL subcellular and extracellular localization by means of confocal microscopy and Western Blot on subcellular fractions lysates and Western Blot on exosomes lysates, respectively. The future studies will be focused on the investigation of TRAIL topology and quantification in TRAIL$^+$ CAFs-released exosomes and on the anticancer activity of these vesicles.

Keywords: CAFs, TRAIL, exosomes

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T04

Interplays between checkpoint kinases, nucleases and helicases in the DNA damage response

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DNA double strand breaks are the most cytotoxic lesions that threat our genome and could lead to genome instability if they are not properly repaired. They are sensed by the protein kinase ATM (Tel1 in yeast), which orchestrates a complex genetic network that arrests the cell cycle through the activation of a checkpoint pathway and repairs the damage [1]. Tel1/ATM represents a relevant target for cancer therapy because either germline or sporadic ATM mutations were identified in different tumors [2]. This project aims at identifying novel synthetic cytotoxic interactions with Tel1/ATM to provide novel targetable proteins in anticancer therapy. We used the budding yeast *Saccharomyces cerevisiae* as a model system and we performed a genomic screening searching on one hand for mutants that exacerbate the sensitivity to DNA damaging agents of cells lacking Tel1, and on the other hand for mutants that can suppress this sensitivity. We identified different mutations in the redundant nucleases Exo1 and Dna2 that either increase or suppress the hypersensitivity to genotoxic stress of cells lacking Tel1, indicating that these nucleases support Tel1 functions in DNA damage response. We then investigated the molecular mechanisms underlying the genetic interactions between Tel1 and nucleases and we propose that these enzymes support Tel1 functions in the recovery of DNA replication when a replication fork is blocked by a DNA alteration.

Keywords:

Saccharomyces cerevisiae, DDR, Tel1, nucleases, genetic interactions

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T05

Integration of omics and functional metabolism data to understand cancer progression rearrangements in bladder cancer advanced *in vitro* models

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Bladder cancer is one of the most common malignancies worldwide. Identifying new markers contributing to patients' stratification is crucial to define more effective and less toxic targeted treatments, improve prognosis, and avoid relapses[1]. Energy metabolism reprogramming is an established cancer hallmark, and altered metabolic pathways can represent attractive clinical targets for new therapeutic strategies[2]. We used 3D cultures, that better simulate the architectural complexity of a tumor mass *in vivo*, to characterize the metabolic and physiological rearrangements induced by spheroid formation in a panel of six bladder cancer cell lines at different stages/grades. Using a systems metabolomic approach, we integrated metabolomics and transcriptomics data, morpho-functional, and metabolic[3] assays with mathematical models of metabolism[4,5]. We show that 3D growth induces a profound stage- and grade-independent gene expression rearrangement indicative of a proliferative rate decrease, differentiation, EMT transition, and correlated with an alteration of metabolic processes e.g. TCA cycle and nucleotides biosynthesis. Moreover, some stage-specific differences accompany the spheroid formation: notably, muscle-invasive cells show a deregulation of lipid metabolism, compared to the non-muscle invasive counterpart. Multi-omics integration could highlight the possible regulatory layers controlling metabolic rewiring in spheroids, to contribute to the identification of novel clinical targets for precision medicine[6].

Keywords: bladder cancer, spheroid, metabolism, high-content analysis, omics integration

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T06

Exploring the interconnections between DNA-RNA hybrids and the DNA damage response

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DNA-RNA hybrids are generated at highly transcribed regions, centromeres and telomeres and participate in physiological processes such as transcription, immunoglobulin class switching and epigenetic modifications[1]. When their homeostasis is perturbed, they can become source of DNA damage and genomic instability, a hallmark of cancer and other genetic diseases. How these structures affect genome stability is not completely understood[2].

This project aims at investigating how DNA-RNA hybrids activate the cellular response to DNA damage and identifying DNA damage response players involved also in the metabolism of DNA-RNA hybrids. Understanding these interplays could be useful for the identification of pathogenesis mechanisms and of new targets and strategies for therapy.

We use the yeast *Saccharomyces cerevisiae* to search for mutations in DNA damage response genes that affect the sensitivity to genotoxic agents of mutants defective in DNA-RNA hybrids processing. We found different mutations in the gene encoding the nuclease/helicase Dna2 that either increase or suppress the DNA damage sensitivity of mutant cells defective for hybrids resolution. These analyses and the measurements of the DNA-RNA hybrids amount in the double mutants suggest that Dna2 participates in DNA-RNA hybrids processing at least when the main pathways involved in this processing are defective.

Keywords: DNA-RNA hybrids, DNA damage, DNA helicases, *S. cerevisiae*

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T07

The challenge of lung cancer early diagnosis: development of a circulating miRNAs signature using liquid biopsy

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Lung cancer (LC) is the second most common cancer worldwide, accounting for 2 million diagnoses per-year. Aggressiveness and the absence of early symptoms lead to high prevalence of non-localized tumors, with subsequent few patients surviving at 5 years (<32%), and a global burden of 1.8 million deaths[1]. Major efforts are therefore focused on identification of biomarkers to increase fraction of early stage and improve survival. Our objective is the development of a clinically-transferable circulating miRNAs signature that will contribute to early detection of LC. Using three published cohorts and application of RankProd non-parametric method[2], we identified 45 circulating miRNAs differentially regulated between 150 tumors and 136 controls. We then profiled (qRT-PCR) the identified panel in plasma samples from 152 LC and 261 controls. Stepwise-logistic regression allowed the reduction of the signature to 9 miRNAs, defining a risk-score able to discriminate between LC and controls, with an AUC of 0.78. To simplify the usage in clinics, we setup a protocol to transfer our signature in digital-PCR. We collected and profiled a large cohort of 259 plasma samples, and focused on nodules ≤30mm which are usually misclassified by CT-scan[3]. We are now applying machine learning techniques for the final definition of the risk-model.

Keywords: early diagnosis; miRNAs; liquid biopsy; lung cancer risk-model

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T08

Caenorhabditis elegans in aging research: characterization of healthspan parameters during lifespan

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One of the main challenges of the 21st century is the progressively aging society: life expectancy has greatly increased in the past few decades, without being accompanied by a similar increment in healthspan[1]. In fact, considering that aging is a time-dependent progressive decline in physiological functions, the consequences of this age-related transition include an increase in frailty and a growing risk of disease[2]. Therefore, research is now focusing on identifying actions to promote healthier aging, rather than just extending lifespan.

C. elegans is a validated model in the field because, besides a short life cycle and evolutionarily conserved pathways, several healthspan parameters can be monitored during aging[3].

Here, I will present how three of these phenotypes change during lifespan in the N2 wild type strain. Briefly, a progressive linear decline of movement and eating rate has been observed since the early adulthood, while resistance to heat stress at 37°C decreases only in the second week of adulthood and it is slightly maintained in the oldest animals. These different trends suggest that features linked to muscle function begin to decrease since the early adulthood, while resistance to stressors is lost later on. ROS levels at days 0, 4, 7 and 14 of adulthood will be analyzed.

Keywords: Aging, *Caenorhabditis elegans*, healthspan parameters

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T09

Synthesis and spatio-temporal imaging of nanoparticles in cultured cells by Ultrafast Electron Microscopy for tumor theranostics

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Ionizable lipid nanoparticles (LNPs) are the most clinically advanced non-viral nano-delivery system for therapeutic nucleic acids (1). The potency of LNPs is testified by the development of patisiran in 2018 and Pfizer/BioNTech and Moderna's mRNA vaccines during the Sars-CoV-2 pandemic (2,3). Despite these successes, several challenges remain in mRNA delivery, including what is known as "endosomal escape". Indeed, reaching the cytosol is mandatory for the therapeutic activity of RNA molecules.

In this project hybrid Lipid/Gold NPs will be synthetized and their cell interactions properties will be characterized through in vitro experiments, such as conventional and innovative light and electron microscopy techniques. Lastly, Ultrafast Electron Microscopy (UEM) will be used to gain further insights in the intracellular trafficking pathways of LNPs.

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T10

Recombinant vault nanoparticle: a potential tool for the targeted delivery of siRNA as therapeutic molecules

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Vaults are eukaryotic ribonucleoprotein particles involved in several cellular processes, with promising application as nano-vector of therapeutic molecules [1]. Here, the yeast *K. phaffii* has been used to constitutively express human recombinant vaults, subsequently purified by size exclusion chromatography. Vault characterization has been conducted by transmission electron microscopy and dynamic light scattering analysis [2]. To promote antibody-mediated vault targeting, a Z peptide-fused vault variant that selectively binds the constant portion of antibodies has been produced. Surface Plasmon Resonance and densitometric analyses have been conducted to determine vault-Z/antibody binding affinity and stoichiometry, respectively. We are working to optimize vault-Z/antibody binding ratio to improve vault uptake. Small interfering RNAs have promising pharmacological potential, which however is constrained by their ability to reach their targets in vivo [3]. Thus, we are currently attempting to load vault-Z with siRNAs targeting LADON, a lncRNA with a role in tumour progression and invasion in melanoma [4]. SiRNAs are loaded into the vaults by chemical conjugation with the interaction domain, which is known to bind tightly vault's inner cavity. Vault complexes carrying anti-LADON siRNAs will then be used to target melanoma cells, where their ability to decrease LADON expression will be tested.

Keywords: vault protein, *K. phafii,* nano-carrier, siRNA delivery, melanoma

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T11

Adverse outcome pathways-oriented toxicology in *in vitro* systems for implementing the safety-by-design of new nanomaterials

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Nanomaterials (NMs) are used in a wide variety of commercial products[1]. Concerns on the potential hazard of these NMs pose questions on their safe development and use in a risk-free framework. The aim of this project is to identify the hazard of new metal-based NMs, designed according to a Safe-by-Design (SbD) approach, during their production and use. A harmonized protocol for the preparation and characterization of the new nanoparticles (NPs) suspensions was applied to evaluate their physical and chemical (p-chem) properties by TEM and DLS. An adverse outcome pathways (AOPs)-oriented testing strategy[2] was applied and the effects of different AgNPs were evaluated on the human lung cell line A549. The results demonstrate that the cellular responses strictly depend on the NPs p-chem properties. In particular, the coating polymers are pivotal in determining the different outcomes. Then, an advanced exposure procedure at the air-liquid-interface (ALI) was developed to expose an *in vitro* co-culture model (alveolar epithelial A549 cell sand macrophage from THP-1 cells) to aerosolized NPs[3] by means of the Vitrocell® Cloud Alpha 12 system. This approach allows for more relevant results than those obtained by submerged culture systems due to a closer mimicking of the human physiology[4]. Furthermore, we used doses of exposure representative of a chronic human exposure estimated considering the data from a monitoring campaign at a manufacturing site working with the selected NPs, and applying the lung deposition model MPPD. Preliminary results obtained exposing the co-culture using the Cultex® system will also be shown.

Keywords: safe by design; adverse outcome pathways; *in vitro* toxicity; inhalation toxicity; air-liquid interface; nanotoxicology.

Acknowledgements: EU-H2020 project ASINA (Anticipating Safety Issues at the Design Stage of NAno Product Development), GA n.862444

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T12

SKIOME project: unveiling the skin microbiome from metadata retrieval to metagenomics meta-analysis with an innovative tool and a curated data collection

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For the past fifteen years, human microbiome research has been a hot topic, leading to countless breakthroughs. Unfortunately, comparing the outcomes of these studies is challenging due to technical differences that can bias results[1]. Therefore, meta-analyses are essential for advancing microbiome science by overcoming these inter-study variations and identifying generalizable trends for a more in-depth understanding[2]. However, data and metadata retrieval from public repositories still represent a challenge for most researchers.

To facilitate and automate data and metadata retrieval process, we designed the bioinformatic tool MADAME (MetADAta MicrobiomE). MADAME is an open-access and user-friendly tool that enables metadata and publications retrieval, and the visualization of these results through report generation before downloading data, enhancing process effectiveness.

However, public repositories' data and metadata are not standardized, requiring researchers to manually curate them before conducting meta-analysis. To contribute to solving this issue, we are updating the SKIOME collection, a curated collection of 16S rRNA amplicon-sequencing skin metagenomics datasets[3]. The collection has the potential to be expanded with additional projects thanks to a novel pipeline. Moreover, it will be available on a website where users will be able to explore statistics and filter metadata.

Our work aims to facilitate multiple datasets integration and, thus, contribute to microbiome research advancement.

Keywords: human skin microbiome, metadata and data retrieval, MADAME, bioinformatics tool, SKIOME collection, 16S rRNA

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T13 FT01 GT08

3D-bioprinted hybrid hydrogels simulating the extracellular matrix dynamics in vitro

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The extracellular matrix (ECM) is a dynamic milieu where aberrant content of proteins, glycoproteins, and GAGs favor tumor growth and invasiveness. It has been also observed that the ECM glycosignature is associate to malignancy [1]. Hyaluronic acid (HA) especially plays a key role in processes linked with significant invasiveness and therapy failure [2].

The production of glycoconjugate biopolymers is a promising route towards tailorable, in vitro systems capable of imitating ECM composition, thus replicating the physical and biochemical properties of the cell microenvironment [3,4].

In this work, HA was crosslinked with various ECM proteins using linkers of varying lengths and branching, generating a library of different ECM mimics. Our purpose is to understand and assess the impact of biochemical and physical behavior in the glioblastoma multiforme (GBM) microenvironment. Therefore, the ECM mimics were tested for three cell lines to prepare an in vitro 3D bioprinted GBM model appropriate for high performance predictive screening and tumor microenvironment research. Furthermore, variations between static and dynamic conditions were investigated applying a flow rate.

Nevertheless, the lack of vascularization is still a significant restriction. To tackle this problem, we prepared a vascularized tissue model using template leaching additive manufacturing hydrogels to replicate a sophisticated and physiologically appropriate vascularized tissue. The tissue model was then evaluated for the successful vascularization of the template leaching channels as well as the functionality of the surrounding tissue [5].

Finally, as cells respond to a wide range of stimuli, we synthetized and functionalized a glycoconjugate hydrogel to better understand the effect of glucose on GBM proliferation and invasion.

Keywords: Bioprinting, Regenerative medicine, Glioblastoma

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T14

Development of a Pressurized Liquid Extraction method for the recovery of glucosinolates from Camelina sativa (L.) Crantz seed by-products and evaluation of their potential bioactivities.

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Camelina sativa (L.) Crantz is a plant belonging to the Brassicaceae family, cultivated mainly for the oil production rich in omega-3 e omega-6 [1]. Following the pressing of the seeds, a seed-press cake by-product remains that could be a source of interesting compounds such as glucosinolates (GLSs). GLS are sulfur-containing glucosidic compounds with potent health benefits[2]. There is an international standard method (ISO9167-1 (Norm, 1992)[3] for the recovery of GLS, however, modern unconventional extraction methods, such as pressurized liquid extraction (PLE) and ultrasound-assisted extraction (USAE), could be a more environmentally friendly alternative that offers many advantages, such as the use of a low amount of organic solvent, higher selectivity, and shorter extraction times leading to an efficient process[4].

The present work aims to develop a green extraction method for the recovery of GLS from C. sativa by-products using AWP. First, the chemical composition of the C. sativa by-product extract obtained with AWP was evaluated by UPLC-HRMS analysis and compared with the ISO extraction procedure, leading to the provisional identification of new GLS. The PLE extraction process was optimized using a chemometric approach, with an experimental design (DoE) to maximize GLS recovery. The results showed that the PLE approach improved extraction efficiency by using less organic solvent than previously developed and optimised ISO and USAE procedures. glucosinolates were purified using a weak anion-exchange solid-phase extraction. Finally, the optimized and purified extracts were tested with *in vitro* assays to assess the potential effects on human health.

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FT08

Heterologous expression of oxidative enzymes in microbial cells for bioconversion of raw materials to products of interest for the pharmaceutical industry

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On 25 November 2020, the European Union adopted the Pharmaceutical Strategy for Europe[1]. This initiative highlights that the delocalization of basic and fine chemicals manufacturing outside the European Union has reached a critical point, where disruptions in the supply chains are no longer mitigated by local manufacturing[2]. Therefore, a considerable effort is being invested to develop local, innovative and more environmentally friendly routes to existing chemicals. This project follows such path as it seeks to find a biocatalytic route to obtain a key building block (5-methylpyrazin-2-carboxylic acid, or MPCA), necessary to the synthesis of molecules used for the treatment of diabetes. The project is focusing on enhancing the efficiency of the chosen pathwaty through the screening of various promoters and via computational screening of potential alternative enzymatic activities.

Keywords: Green economy, biorefinery, metabolic engineering

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FT09

Metabolomics approach coupled with multivariate analysis of different parts of Prosopis cineraria (Ghaf) for evaluation of their antioxidant activity

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Nowadays noncommunicable diseases (NCDs), also known as chronic diseases, are the cause of the 74% global deaths each year. Among them, the main types of NCDs are those related to cardiovascular disorders, cancer, chronic respiratory diseases, and diabetes. In a word where the non-communicable diseases are even more increasing their incidence and where the medicine struggles to keep up with effective pharmaceutical treatments or these are too expensive, the prevention could play a key role [1]. The 21st century is characterized by climate change, temperature rises, drought and lack of water. These factors impact on agronomical production hindering both economical and human health related aspects. In this context the investigation of tropical and subtropical plants as potential sources of high added values products can play a key role. Here we propose a study on *Prosopis cineraria* (L.) Druce*,* commonly known as Ghaf, a tropical plant from the Fabaceae family (sub. Mimosaceae) able to grow in arid and semi-arid environments; in 2008, this tree has drawn attention for its various uses, and it was declared as the national tree of the United Arab Emirates (UAE) [2].These characteristics, along with its documented therapeutic properties make it a promising matrix for multiple bioprospecting applications for human health purposes [2]. Metabolomic analysis is an innovative approach to investigate natural matrices, integrating complex data from chemical identification as mass spectrometry profiling and biological response. The present study aimed to carried out comparative chemical composition of different part of Ghaf, such as leaves, roots, twigs and bark. All matrices have been extracted with different solvents and the chemical composition determined by GC-MS and UHPLC-HRMS QTof. The obtained data were also combined for their *in-vitro* antioxidant activity by Multivariate Data Analysis to define a comprehensive fingerprint of the Ghaf and to identify the most promising bioactive components of this plant that could be used for the formulation of nutraceutical or pharmaceutical products. Preliminary results suggest that *P.cineraria* can be considered an interesting source of bioactive compounds useful in disease prevention and health promoting effects.

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FT10

NO PAINs NO GAINs: the importance of TR-FRET interference assays for the validation of true-positive hits

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Unidentified Pan-Assay Interference Compounds (PAINS) may compromise hit discovery programs based on high-throughput screening aimed at identifying novel molecular classes of compounds for potential therapeutic intervention. In this perspective, the importance of GAINS[1] (give attention to limitations in assays) was introduced and highlighted their relevance for a successful drug discovery program.

To support and complement the primary assay, based on a protein-DNA interaction which underwent high-throughput screening process, we present an approach utilizing interference assays, designed in agreement with the primary assay, based on Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) to validate true-positive hits[2].

We focused on the development of a peptide-based TR-FRET interference assay as a valuable tool to support the protein-DNA primary assay in highlighting and discriminating compounds with non-selective nature. Having a robust and accurately developed interference assay is crucial for ensuring good reliability of screening results in detecting interfering compounds.

In conclusion, by implementing these assays early in the drug discovery process, researchers can streamline the identification of promising candidates and expedite the hit identification process.

Keywords: Drug discovery, High-throughput screening, False-positive hits, Interfering compounds, Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET), Protein-DNA interaction

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FT11

Advanced biomaterials engineered for the production of sustainable compounds

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Cellulose is the most abundant polymer in nature, present both in plants and microorganisms biomasses, and has long been a major renewable source of material[1]. This linear polymer is made up of β-1,4-glycosidically linked anhydroglucose monomers, with three reactive hydroxyl groups on each unit. Their further modification, thus introducing new functional groups, is necessary for specific applications[2]. In particular, a modified cellulose can be incorporated in natural rubber compound as filler system, to replace silica and carbon black and reduce the environmental impact of the composites themselves throughout their life cycle[3].

Chemo-enzymatic oxidation can be addressed with the Laccase-mediated system (LMS): in the presence of the (bio)catalyst laccase, TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical) acts as mediator oxidizing the final substrate, cellulose[2,4]. Those reaction conditions have been optimized at different temperatures and pH. Regarding the techniques applied, spectrophotometric analyses has been performed for the evaluation of the enzyme activity, infrared spectrometry to identify in a qualitative way if the cellulose samples have been modified[2] and finally a quantitative method that takes advantage from the use of triphenyltetrazolium chloride (TTC) reagent[5] has been implemented to determine the degree of cellulose functionalization.

To conclude, the hydroxyls transformation into oxidized functionalities (like carbonyl) set anchoring sites for subsequent steps.

Keywords: biomaterials, cellulose, biocatalysis, tyre

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FT12

Assessment of ecosystem pollination service in Italian urban environments: first results and future perspectives

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In the last decades, a global decline in biodiversity has been taking place due to the strong anthropogenic impact [1]. However, in large cities, refuge areas for pollinator communities persist[2] and from a one-health and biodiversity perspective it is necessary to protect and implement suitable green areas to support functioning ecosystems[3].

To detect pollination service in urban green spaces, monitoring activities were implemented in 12 sampling areas for each of six major Italian cities, selected according to different size and fragmentation in relation to the surrounding urban matrix. These activities yielded data on diversity and abundance of important pollinators such as wild bees and hoverflies and it will provide valuable information on plant-pollinator interactions, which characterize the pollination ecosystem service. This took place within the activities of spoke 5 (Urban Biodiversity) of the PNRR and NBFC plan, from May to July 2023, monthly, collecting over 4000 wild bees and hoverflies specimens.

A comprehensive review of the literature about actions and monitoring activities in urban areas to improve pollinators also is being carried out. Furthermore, data on the relationship between the mature urban forests and pollinators' diversity and efficiency, has been conducted by processing previous data available on pollinator communities in Milano.

The amount of data being produced at the moment during my PhD will shed light on the complex relationship between green areas and the regulating ecosystem services towards a more sustainable and healthy urban environment.

Keywords: pollinators, urban biodiversity

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FT13

Phylogenetic analysis for research of plant active compound

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Each plant has its own wide range of secondary metabolites, these molecules can exert different effects that may be useful in treating different human disease. However herbal drug discovery is expensive and time-consuming, so it is necessary to efficiently choose plants for the research of these compounds. One approach to identify plants with potential useful molecules is the phylogenetical one, as closely related plant species tend to share biochemistry and medicinal properties 1,2. The aim of this project is to apply comparative phylogenetic methods in finding plants containing potential molecules usable in the development of new drugs. For the application of these methods, information about plants with a known effect on a disease was combined with information about species relationship on a phylogenetic tree. Different statistical analyses were first used to measure the presence of phylogenetic signal and then to identify the plants found in hot nodes 3. At last, the probability of having an effect on the disease was calculated for each plant found. This work was able to give a better understanding on the use and potential of phylogenetic methods in finding the most promising plants to study for a disease drug discovery.

Keywords: bioprospecting, phylogenetics, medicinal plants

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FT14

UniBiome project: new microbiome-inspired approaches for a sustainable urban regeneration of Universities

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During the last decades, a consistent rise of non-communicable diseases has been registered, and one of the leading causes is the reduction in the biodiversity of the human and environmental microbiome[1,2]. The immediate future challenge is to inflect the urban regeneration wave to make it sustainable from every point of view, including the microscale. How can we transform modern infrastructures into health-promoting buildings? From a microbiome-oriented perspective, the project aims to evaluate the salubrity of two Italian Universities - the University Milano-Bicocca and the Politecnico di Milano - integrating a Student-Science approach in the project design. More than 150 students have been sampled (both skin and gut microbiome samples), while from the outdoor and indoor areas of the two campuses, more than 500 surface and soil samples have been collected from bathrooms, classrooms, canteens, squares, etc. The university microbiome will be characterized with amplicon-based sequencing and bioinformatics analysis will be performed. Exploiting the role of microbial functional biodiversity, guidelines for a bio-informed renovation of the campuses will favor students' and the ecosystem's health[3].

Keywords: Built Environment, Microbiome, Urban Regeneration, Urban Health

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T15

Role of glycoside hydrolases family 3 in Antarctic marine bacterium

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Cold environments constitute a widespread habitat on planet Earth. Here, organisms able to survive are the so-called psychrophiles, that developed a variety of evolutionary adaptations to suit this extreme condition. The expression of cold-active enzymes is one of the main cold adaptation strategies adopted by psychrophilic organisms to counteract the harmful effects of cold environments at molecular level. Cold-active enzymes have peculiar structural and functional features which makes them of industrial interest [1].

β-glucosidases are ubiquitous glycoside hydrolases, found in all domains of living organisms. These enzymes act by cleaving the β-glycosidic bonds present in polysaccharides. Among polysaccharides, marine polysaccharides are a heterogeneous group, differing in composition and complexity. β-glucosidases active on marine polysaccharides are therefore a useful biotechnological tool for exploiting the full potential of marine polysaccharides [2,3].

Here, we report the characterization of two enzymes belonging to glycoside hydrolases family 3, identified in the genome of *Marinomonas* sp. ef1, an Antarctic marine bacterium. These two enzymes, namely M-GH3A and M-GH3B, show different biochemical features, structural characteristics, and substrate specificity. Furthermore, they seem to be active on marine polysaccharides, suggesting that they may play a different role in the degradation of polysaccharides or oligosaccharides of marine origin.

Keywords: cold-active enzymes, beta-glucosidases, polysaccharides

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T16

Safe and sustainable nano-enabled antimicrobials to reduce the presence of contaminants of emerging concern in the aquatic environments: a focus on CuO-based nanomaterials

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Antimicrobial resistant (AMR) bacteria represent a class of contaminants of emerging concern that can be found in water bodies as a result of anthropic activities. Nanoparticles (NPs) and nano-enabled products emerged as novel effective antimicrobial agents against such contaminants[1]. Nevertheless, their safety and sustainability must be evaluated early in the innovation process[2]. The increase in nanomaterials (NMs) manufacture and use may lead to inappropriate disposal into the aquatic environment, representing a potential risk to non-target species[3]. Furthermore, there is high uncertainty about the impacts of NMs on the environment during their life cycle[4]. CuO NPs have been extensively used as bactericidal agent[5], yet their potential toxicity to cells and organisms is recognized[3]. This work aimed at evaluating the safety and sustainability of novel CuO-based NMs produced to face aquatic AMR bacteria. The nanosafety was evaluated in exploitation scenarios by using the model organism zebrafish (*D. rerio*). The aquatic toxicity potential was assessed by the Fish Embryo acute Toxicity (FET) test (OECD n. 236), while activity-related parameters of zebrafish embryos were analysed with the DanioScope Software to investigate additional sublethal effects. A life cycle assessment (LCA) approach was implemented to evaluate the environmental impacts of the NMs' production, so to identify the most critical aspects at this stage of the life cycle.

Keywords: antimicrobial nanoparticles, CuO, zebrafish, aquatic toxicity, behaviour, safe and sustainable, Life Cycle Assessment

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T17

Optimization of synthetic biology tools to increase modularity for the production of natural dyes in *Saccharomyces cerevisiae*

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The current fashion system is based on a non-sustainable linear economy model. In this context, natural colorants can have a role in the shift towards a more circular economy model, as an alternative to fossil-based synthetic dyes[1]. Among natural colored molecules, some hydroxyanthraquinones (HAQ) from plants and fungi show qualities desirable for textile dyeing (e.g. high light-fastness and low toxicity)[2].

Questin and skyrin, respectively yellow- and red-colored, were selected as target HAQ for the heterologous production in *Saccharomyces cerevisiae*[2]. A first set of engineered strains was created for the biosynthesis of emodin, the common colored precursor of the two molecules, however, none of them was able to produce a detectable product. Fluorescence microscopy allowed us to identify a possible bottleneck in the functional expression of the first enzyme of the pathway, implying the need to partially redesign the strains in a new design-build-test-learn cycle.

The available synthetic biology toolkits still lack modularity at the strain level, making the modification of the already extensively engineered strains difficult, especially when changes in elements introduced in the earlier engineering stages are needed. Thus, a second output of this work was the development of a novel synthetic biology strategy to bring modularity at the strain level, making the strains' (re)building more flexible and allowing to speed up the synthetic biology design-build-test-learn cycle.

Keywords: yeast, natural dyes, hydroxyanthraquinones, synthetic biology, modularity

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T18

From Pollinator Diet to Human Nutrition: the surprising insights from pollination ecology

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Landscape anthropization is recognized as the primary driver behind the decline of insect pollinators, ultimately leading to the loss of pollination ecosystem service [1]. In this context, unraveling the specific pressures affecting insect pollinators and determining effective management strategies is of utmost importance, as pollinators health is intricately intertwined with ecosystem integrity and human well-being. In our study, we adopted a comprehensive multi-level integrative approach to investigate the potential effects of landscape anthropization on pollinators diet. We characterize the nutritional profile of the pollen foraged by *Bombus terrestris* along an urbanization gradient and concurrently we conducted an analysis of the chemical composition of floral rewards (i.e., pollen and nectar) of different species of wildflowers. Furthermore, we sought to assess the link between pollination service and human nutrition through an experimental design encompassing two agricultural crops (i.e., *Fragaria vesca* and *Vigna unguiculata)*. Specifically, we investigated differences in commercial quality and nutritional profiles of fruits and seeds under various pollination treatments. The results obtained highlighted a strong role of habitat in the determination of pollinator nutrition with marked variations in macronutrient content in foraged pollen and alterations in the chemical profile of floral rewards along the anthropization gradient. Notably, the pollination treatments applied in our experiment revealed a significant difference with insect-pollinated fruits exhibiting higher commercial and nutritional quality.

Collectively, these findings underscore the interconnectedness of environmental preservation, biodiversity conservation, and human health that aligns with the holistic principles of the One Health concept.

Keywords: Insect pollinators, Phytochemistry, Nutritional ecology

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T19

Implementation of a bioeconomy strategy in the textile industry

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Due to its linear economic model, the textile industry is one of the most relevant for its environmental impacts[1]. The overproduction of pre- and post-consumer waste has a significant environmental impact. Only about 1% of textile waste is recycled globally, with 5.8 million tons ending up in landfills in the EU [2].

The aim of the project is to develop a circular economy strategy that allows for the pursuit of the "zero waste" principle by exploiting side streams and building blocks released from waste hydrolysis to produce new molecules of interest within the production chain via microbial biotransformation processes, thereby improving industrial synergy and bioeconomy[3].

The first goal of the project was to investigate an approach for the valorisation of waste from the textile manufacturing production sites. The strategy comprises a combination of treatments, exploiting a second residual NaOH-rich effluent from the finishing process waste and enzymatic hydrolysis, resulting in an optimized glucose release with yields ≥ 85%.

Recently, Albini Group, one of the world's most relevant companies for the manufacturing of natural fibres fabrics, in partnership with the start-up Colorifix, collaborated to implement a new biobased industrial process for yarn dyeing. Thus, the second goal of the project was to implement the machinery required and to develop the industrial fermentation process via microbial cell factories for the in-house production of dyes for their use in Albini's industrial dyeing process. The project led to the implementation of sustainable dying technology, aiming at the evaluation of all the phases of the process pipeline, from feasibility study through industrial scale-up, opening to the bioeconomy business model, and applying the biorefinery principles.

Keywords: Textile waste, Hydrolysis, Circular Economy, Microbial biotransformation, Bioeconomy.

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T20

Yeast fermentation for the upcycling of PET monomers

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Plastic has become an indispensable material in many fields, with production increasing every year; however, most of the plastic waste is still incinerated or landfilled, and only 10% of the new plastic is recycled even once. Among all plastic, polyethylene terephthalate (PET) is the most produced polyester worldwide (56 Mt/year).

This work focuses on the upcycling of PET monomers – terephthalic acid (TPA) and ethylene glycol (EG) – by yeast fermentation for the production of industrially relevant organic acids (protocatechuic acid, *cis,cis*-muconic acid, 3-carboxy-*cis,cis*-muconic acid, glycolic acid) and on the use of EG as a carbon source.

For the bioconversion of TPA, several heterologous genes were introduced, including potential TPA transporters.

To characterize the native catabolism of EG, strains overexpressing and with deletions in key genes of the pathways were assayed; in parallel, two synthetic metabolic pathways are being introduced in *S. cerevisiae* for a more efficient EG assimilation. A DoE approach is ongoing for the optimization of glycolic acid production. *In silico* constraint-based models are currently being developed to optimize growth and production on TPA and EG.

Our research shows promising results and intriguing challenges in the biodegradation and upcycling of PET monomers by yeast fermentation, and it will become even more interesting thanks to the recent advances in enzymatic PET hydrolysis.

Keywords: *Saccharomyces cerevisiae*, Polyethylene terephthalate (PET), Bioconversion, Synthetic biology, Bioprocess engineering

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T21

Exploring Thermodynamic Properties of Biomolecules: Case Studies of FLUC Ion Channel and Flavodoxin Redox Enzyme through High-Performance Computing-based Simulations

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Computing the free energies to gain insights into the behavior of biological systems still represents a significant challenge in biotechnology field[1, 2]. Here it is reported two case studies in which are used different advanced simulation approaches to explore the thermodynamic properties of ion channels and redox enzymes. The first project is focused on flavodoxins, enzymes that play an important role in energy conversion and electron transfer[3]. The purpose of this work is to verify whether it is possible to predict the redox potentials within protein systems combining non-equilibrium thermodynamic integration approach and molecular dynamic simulations[4]. The implications could result in the ability to study electron transfer in biotechnologically relevant complexes and the ability to fine-tune such property in a bio-molecular devices. The second project investigates Flucs, ion channels responsible for expelling fluoride ions from bacterial cytoplasm[5, 6]. The study combines experimental data with hybrid QM/MM molecular dynamics simulations, utilizing the MiMiC interface and metadynamics techniques[7]. This approach allows for the modeling of large membrane protein systems, providing molecular-level insights into the ion permeation mechanism and selectivity of Flucs. The simulations aim to elucidate the binding sites and roles of specific residues in fluoride recognition, furthering our understanding of these ion channel selectivity. Both projects pointes out the significance of in silico simulations in unraveling complex thermodynamic properties by calculating free energies, contributing to advancements in fundamental and applied research.

Keywords: Redox Potential, Flavoprotein, Molecular Dynamics, Thermodynamic Integration, Fluc, Anion channel, QM/MM, MiMic.

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T22

New perspectives for a formulation company: from blending to sustainable chemical manufacturing.

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Bulk chemicals like small esters are widely used compounds in many industrial applications as solvents or intermediates[1,2]. Companies such as Liberty Chemicals and Vertec Biosolvents are currently expading the use of esters in established industrial applications. As a matter of fact, the right blend of small esters can be used to replace aromatic and paraffinic solvents in the formulation of inks and coatings or in industrial cleaning procedures with enourmous benefits in relation to human and environmental toxicity. Even dought it is theoretically possible to produce some small esters from bio-based alcohols and acids in industrial practice molecules, like ethyl acetate, are still produced mainly through unsustainable chemical processes, with energy intensive procedures and starting from fossil-based feedstocks[1,2]. The project aims to develop an industrially viable biotechnological route for the production of small esters starting from renewable resources with a view to circular economy and carbon neutral balance[1]. Results obtained so far are very encouraging and currently we are in the phase of submitting a patent application to protect our technology.

Keywords: bulk chemicals, LMW esters, ethyl acetate, biocatalysis, fermentation

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T23

Discovery of new potential antibiotic molecules from a rare actinomycetes genus

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Antibiotic resistance has gained considerable interest over the years. Nonetheless, antibiotic resistance is not the only way for bacteria to escape antibiotic treatment. Since very few antibiofilm drugs are available [1], biofilms – which are made of surface-adhering bacterial communities – too are seen as a threat to public health [2]. The aim of the project is to discover new antibiotics with potential antibiofilm activity, by characterizing *Actinobacteria* strains belonging to a rare and understudied genus, *Microbispora*, from the Naicons library by paired omics profiling as previously described.[3]

At first, 166 strains contained in our library belonging to this so-called rare genus were identified and characterized by 16S rDNA sequence analysis. Culture conditions and extraction methods that allowed for high molecular diversity in strains extracts were also determined. Then, a MS fingerprints library was generated from the 498 obtained extracts. In parallel, the genome of these strains was sequenced, allowing us to get a library of Biosynthetic Genes Clusters.

Combining antibiotic and antibiofilm activity screening with metabolomics and genomics tools to analyze these datasets, we were able to characterize the metabolome of our strains and we are identifying and characterizing molecules that are potentially bioactive.

Keywords: drug discovery, actinomycetes, paired omics, antibiotics

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FT15

Contribution of the Wiskott-Aldrich syndrome protein's functions of microglia and brain resident macrophages in physiological and pathological conditions

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The Wiskott-Aldrich syndrome (WAS) is a rare disease caused by mutated WAS gene. The WAS protein (WASp) is involved in hematopoiesis and phagocytosis by immune cells[1]. WAS patients develop immunodeficiency, but 40% of them show neurological manifestations[2], whose basis is unknown. We hypothesize that neurological deficits in WAS are in part due to impaired myeloid-cell mediated brain development or homeostasis keeping. In the brain, WASp is expressed by microglia/macrophages, which participate to neurodevelopment and neuroinflammation through their phagocytic behavior[3,4,5]. We obtained, from human induced pluripotent stem cells (hIPSCs), microglia (iMicro) and macrophages (iMac), overexpressing the selective microglia marker *P2RY12* (4.23±0.64 Log_2 fold-change±SD vs. progenitors) and macrophage *CD68* (1.11±0.73) and *CD163* (2.43±0.99). iMicro expressed *WAS* (2.32±0.52), while iMac downregulated it (-6.73±2.92). After LPS inflammatory hint, iMac expressed again *WAS*, similarly to their progenitors (-0.91±0.50). By a confocal time-lapse phagocytic assay on iMicro overexpressing *WAS*, they appeared phagocytic, a function prevented by the WASp inhibitor Wiskostatin (109.40±12.72 vs. 54.38±3.94 AUC±SD CTRL vs. Wiskostatin). In conclusion, *WAS* is expressed by microglia and by stimulated brain macrophages. In microglia, WASp is needed for phagocytosis, a key observation for next studies aimed at clarifying how WASp impacts the phagocytosis-mediated synaptic pruning during physiological brain development.

Keywords: primary immunodeficiency, human Induced Pluripotent Stem Cells, brain's resident immune cells, brain development

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FT16

Reactivation of miR-29 to Mitigate Tumor Adaptation and Hormone Resistance in Prostate Cancer

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Prostate cancer is the second most common cancer in men, causing more than 350'000 deaths annually.[1] The emergence of acquired resistance to treatments significantly undermines the success of therapeutic interventions.[2] Lineage plasticity, which refers to the ability of cells to change their phenotype, plays a crucial role in cancer evolution and treatment failure.[3] Analysis of transcriptomic profiles obtained from normal prostate tissue, primary tumors, and metastatic disease has revealed an aberrant reactivation of chromatin-modifying enzymes involved in reshaping the epigenetic landscape in castration-resistant tumors. Specifically, we observe a significant upregulation of DNA methyltransferases (DNMTs) and DNA or histone demethylases (TETs/KDMs), most of which are direct targets of miR-29. During tumor progression, miR-29 family members are downregulated and their repression could contribute to the development of hormone independence. Accordingly, we hypothesize that miR-29 deficiency may drive therapy adaptation through the enhancement of cellular plasticity due to epigenetic alterations.[4] Therefore, it becomes relevant to investigate whether modulating miR-29 expression could restore hormone dependence and increase tumor sensitivity to androgen depletion.

Keywords: Prostate Cancer, Micro-RNA, Epigenetics, Plasticity.

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FT17

Characterization of plant bioactive molecules in eukaryotic models of Parkinson's disease

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Plants are historically recognized as a source of bioactive molecules useful for several applications in the fields of medicine, cosmetics and food industry. In recent years, the protective effects of these bioactive compounds have been highlighted on a wide variety of diseases among which diabetes, cardiovascular and neurodegenerative diseases [2].

Aging and age-related neurodegenerative disease are among the main challenges in modern medicine [3] because the recent increase in human lifespan hasn't corresponded to a healthy longevity. Nowadays, Parkinson's disease, which is associated with misfolding of ⟨-synuclein protein, affects about 10 million people worldwide [1].

This PhD project, belonging to the main topics of the Italian National Center of Biodiversity, aims to identify plant-based bioactive molecules of the Mediterranean area, considering their potential antioxidant, anti-aging and neuroprotective properties. To find high added value in endemic plants, eukaryotic models expressing human ⟨-synuclein have been taken into consideration.

To achieve these goals, we are using budding yeast cells overexpressing ⟨-synuclein to assess the effects of a number of dry extracts on cellular longevity, ROS levels and ⟨-synuclein's toxicity. Moreover, to evaluate cellular metabolism, the induction of catabolic processes such as autophagy and protein degradation are also under investigation.

Keywords: bioactive molecules, Parkinson's disease, neurodegeneration, protein aggregation, senescence, anti-oxidant effect

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FT18

Intracellular Ca^{2+} dynamics modulation by istaroxime and its metabolite in pulmonary artery smooth muscle cells

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Smooth muscle cells (SMCs) can undergo phenotypic changes in response to vascular diseases, affecting Ca^{2+} handling proteins, such as SERCA and store-operated Ca^{2+} entry (SOCE) related proteins [1]. In cardiac preparation, istaroxime is a luso-inotropic agent able to inhibit the Na^+/K^+ ATPase and stimulate SERCA2a activity, while its metabolite (PST3093) is a selective SERCA2a stimulator [2].

The aim of the project is to investigate the effects of istaroxime and PST3093 on rat pulmonary artery SMCs (rPASMCs) intracellular Ca^{2+} dynamics.

rPASMCs were characterized for the α-SMA, SERCA and phospholamban (PLN) expression levels. Intracellular Ca^{2+} dynamics were assessed by using Fluo4-AM or Fura2-AM dyes, evaluating SOCE, ATP-induced sarcoplasmic reticulum (SR) Ca^{2+} release and resting Ca^{2+} level. Finally, Na^+/K^+ ATPase current (I_{NaK}) inhibition was evaluated in V-clamped rPASMCs. In cultured rPASMCs, SERCA2b was the main expressed SERCA isoform; PLN was not detectable. Istaroxime (100 nM) reduced resting Ca^{2+} and SOCE, while it did not affect the amplitude and kinetic of ATP-induced Ca^{2+} transient; I_{NaK} inhibition potency was lower in comparison to cardiac preparations. All these effects were not shared by PST3093.

These results suggest that istaroxime affects rPASMCs Ca^{2+} dynamics through mechanisms not related to SERCA2a stimulation and potentially related to SOCE inhibition.

Keywords: SMCs, istaroxime, PST3093, Ca^{2+} handling, SERCA, SOCE

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FT19

The impact of Rai1 haploinsufficiency on lipid metabolism and autophagic flux in Smith-Magenis Syndrome

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Smith-Magenis syndrome (SMS) [1] is a neurodevelopmental disorder that is currently incurable and difficult to diagnose, characterized by physical, metabolic, behavioural, cognitive, and sleep-wake cycle alterations. SMS is caused by haploinsufficiency of the RAI1 gene, due to both a deletion of the short arm of chromosome 17 (17p11.2) or a mutation within the RAI1 gene.

The roles of RAI1 and the molecular mechanisms leading to the onset of the disease are still largely unknown.

Here we study the impact of RAI1 haploinsufficiency on autophagy flux, through immunostaining for LC3 puncta [2], lipid metabolism, via Oil Red O and Bodipy$^{493/503}$ tests [3] and lysosome homeostasis, through LysoTracker kit and immunostaining for lysosome markers.

We are also starting to test some molecules, such as N-acetylcysteine (NAC), with the aim of reversing the phenotype.

Keywords: SMS, LD, LC3, NAC.

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FT20

Primary cilium characterization in Joubert Syndrome somatic cells

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The Joubert Syndrome is a rare neurodevelopmental disease belonging to the family of ciliopathies. About 30 genes have been identified to be causative of the disease when mutated, and all of them encode proteins involved with the structure or function of the primary cilium. This organelle is present in most of the cell population and acts in detecting environmental changes and transducing many signals. This project aims to characterize the effects of mutations on the protein Jouberin, codified from the *AHI1* gene, responsible for Joubert Syndrome 3 *(1)*. Primary fibroblasts were collected from two patients carrying homozygous mutations on the WD40 domains of *AHI1.* Both lines were characterized, revealing alterations in the proliferative rate with respect to healthy controls. To study the primary cilium structure, fibroblasts were starved, to promote ciliogenesis. The mutated cells exhibited an altered timing during cilium assembly and disassembly and differences in the aspect of the primary cilium with respect to the controls. In particular, Joubert fibroblasts presented longer cilia, higher levels of acetylated tubulin along the axoneme, and a delay during the disassembly process *(2)*. Thus, suggested that mutations in Jouberin affect somehow the correct deacetylation pathway involved with the primary cilium disassembly.

Keywords: Joubert Syndrome, primary cilium.

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FT21

Multidimensional data integration to capture intrinsic and extrinsic mechanisms driving treatment benefit in breast cancer

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In the last few decades precision oncology developed personalized treatments for breast cancer based on molecular biomarkers. This strategy has remarkably improved overall patients' outcome and decreased mortality[1]. The most important biomarkers are the expression of estrogen receptor (ER), progesterone receptor (PG) and the amplification of the Human Epidermal Growth Factor Receptor 2 (HER2).

However, treatment tailoring is still far from being truly personalized because of the onset of drug resistance and the excessive overtreatment which cause toxicity to patients.

"Omics" technologies increase our ability to characterise the tumour and its microenvironment at genomic, transcriptomic and proteomic level, but there is a huge gap between the amount of information available and its translation into clinically useful biomarkers[2]. To understand the resistance mechanisms and identify the most appropriate treatment for patients, investigation of tumoral heterogeneity is needed. The project aims to develop a framework to integrate multi-dimensional molecular data, building upon state-of-art understanding of solid tumour biology and studying both tumour- and microenvironment-related features, to provide a more informative and complete portrait of the tumour as a system and to develop predictors of response to established and emerging treatments for breast cancer, including chemotherapy and immune checkpoint inhibitors.

Keywords: breast cancer, biomarkers, data integration

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T24

Unveiling dietary habits in elderly: hippuric acid and food-derived markers in frailty

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Frailty is a geriatric syndrome characterized by a decrease in the physiological reserves, leading to higher vulnerability to stressors. Due to the established association between dietary patterns and risk of frailty[1,2], the identification/measurement of food-intake biomarkers (FIBs), molecules derived from food ingestion and metabolism, is crucial for an objective information on dietary habits.

We set-up an untargeted mass spectrometry-based nutrimetabolomic workflow to identify FIBs for investigating the association between diet and frailty in plasma samples from 130 elderly subjects classified as Fit or Frail (InveCe.Ab population).

To assess the correlation/association between dietary habits and food metabolism, identified FIBs were assembled into food categories and used to compute a Mediterranean Diet Score that incorporates nine dietary components (beneficial/detrimental) from food questionnaire.

FIBs categories showed high prevalence of consumption in vegetables, meat, coffee, tea, cereal, legumes and low prevalence in fish/seafood, alcoholic beverages, olive oil in all subjects. When FIBs were individually tested in relation to frailty, significant abundance differences in hippuric acid, quercetin, caffeic acid and isorhamnetin were found between Fit and Frail. In particular, hippuric acid abundance, that is negatively associated with frailty, was assess as predictor of frailty onset by a targeted quantification in the InveCe.Ab longitudinal population.

Keywords: Nutrimetabolomics, Frailty, Mass spectrometry, Food-intake biomarkers, Hippuric acid

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T25

The combination of the PPARγ agonist pioglitazone and the RXR agonist IRX4204 induces adipocytic differentiation in myxoid liposarcoma

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Myxoid liposarcoma (MLS) is characterized by an adipogenesis block that causes the accumulation of immature adipoblasts with uncontrolled proliferation. Trabectedin reactivates adipogenesis but, although it has good efficacy in MLS patients, resistance occurs with no further effective therapies available[1]. Recent data show that the PPARγ agonist pioglitazone reactivates the adipogenic pathway in MLS preclinical models resistant to trabectedin, restoring trabectedin efficacy[2]. Since PPARγ heterodimerizes with the retinoid X receptor (RXR), one possible strategy to further enhance adipocytic differentiation is to combine trabectedin and pioglitazone with the RXR agonist IRX4204[3]. In this study, drug's efficacy was assessed in MLS patient-derived xenografts ML017 (sensitive), ML017/ET and ML006 (resistant to trabectedin). Mice received pioglitazone (150 mg/kg p.o. qdx28), trabectedin (0.15 mg/kg i.v. q14dx2 or q14dx3), IRX4204 (10 mg/kg i.p. qdx28) or their combinations. We found that IRX4204 in combination with trabectedin and pioglitazone improved their antitumor activity, causing faster tumor growth inhibition. In addition, a prolonged tumor growth inhibition was observed in ML017 and ML017/ET models by combining IRX4204 with pioglitazone, even without trabectedin. This last result might represent a clinically relevant opportunity, especially for patients not suitable to receive trabectedin.

Keywords: myxoid liposarcoma, trabectedin, RXR agonists, patient-derived xenografts

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T26

Identification of new therapeutic strategies for mucinous ovarian carcinoma using CRISPR/Cas9 libraries

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Mucinous ovarian carcinoma (mEOC) is the rarest subtype of epithelial ovarian cancer, it is particularly aggressive and poorly responsive to chemotherapy[1]. Our recent research has identified PLK1 (Polo-like kinase 1) as a druggable target in mEOC[2]. In this study, our objective is to identify potential synthetic lethality partners of PLK1 using CRISPR libraries. Firstly, we established three distinct mucinous cell lines (MCAS, EFO, and TOV) expressing the Cas9 enzyme, whose functionality was verified through a GFP assay. For the screening experiment, were utilized two Bassik gRNA libraries[3]: Human CRISPR Deletion Library - Apoptosis and cancer (Addgene #101926) and Human CRISPR Deletion Library - Drug targets, kinases, and phosphatases (Addgene #101927). The multiplicity of infection of viruses derived from these libraries was calculated prior to the screening experiment, which was performed in EFOCas9 cells, both with and without a PLK1 inhibitor (Onvansertib), enabling the identification of genes essential for mucinous cells and genes exhibiting synthetic lethality in combination with the PLK1 inhibitor.

Preliminary results have yielded a set of candidate genes that require further exploration and functional characterization. These findings could contribute to a deeper understanding of the biology of mucinous ovarian carcinoma and aid in the development of novel treatment strategies.

Keywords: Mucinous ovarian carcinoma, CRISPR libraries, PLK1 inhibitor, synthetic lethality, druggable targets.

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T27

Strategies to overcome olaparib drawbacks: from patient selection to novel therapies

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Poly(ADP-ribose)polymerase inhibitors (PARPi) have changed ovarian cancer management and are particularly effective in homologous recombination defective (HRD) tumours.[1] To select patients eligible for therapy, clinicians employ genomic scarring tests and *BRCA1/2* mutations as surrogate markers of HRD.[2]

In my project, I exploited 20 ovarian carcinoma patient-derived xenografts (OC-PDXs) to assess **i)** HRD testing predictability on PARPi response and **ii)** the efficacy of a novel PARPi more selective for PARP1 and less toxic.[3]

The OC-PDXs chosen represent the clinical scenario with 80% of high-grade serous OC; 90% mutated in *TP53* and 50% in *BRCA1/2* genes. 10% of tumours lack *BRCA1* expression. HRD testing classified 70% of OC-PDXs as positive based on score ($\geq$42) and/or *BRCA* mutations.

Olaparib was tested in 18 OC-PDXs and 50% appeared to be sensitive. Importantly, 30% of tumours classified as HRD-positive were olaparib resistant. Conversely, all HRD-positive OC-PDXs were sensitive to the novel PARP1-selective inhibitor.[4] Besides being effective on olaparib resistant tumours, it had improved tolerability when combined with carboplatin, a standard-of-care treatment for OC.

In conclusion, we found that HRD testing has a low negative predictive value for olaparib response and that the novel PARP1-selective inhibitor represents a promising option for therapy, offering new opportunity for combination strategies.

Keywords: Ovarian Carcinoma Xenografts, PARP inhibitors, HRD testing

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T28

Ovarian cancer and PARP inhibitors: the emerging role of mitochondrial metabolism

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The introduction of Poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitors has transformed the therapeutic approach of ovarian cancer (OC) [1]. These drugs target homologous recombination (HR) deficient cancer cells through synthetic lethality, while sparing HR proficient cells [2]. Evidence sustains the existence of an interplay among PARP activity, DNA repair and mitochondrial metabolism [3], which has been recognized to play a key role in OC malignancy [4; 5; 6].

The aim of my project is to investigate how the mitochondrial metabolism influences the response to PARP inhibitors. To this purpose, I exploited both a pharmacological and a genetic approach to impair mitochondrial metabolism, employing an inhibitor of the electron transport chain and genetically modified OC cell lines.

I observed that the impairment of mitochondrial metabolism is associated with increased sensitivity to PARP inhibitors that is associated with an accumulation of double strand breaks (γH2AX foci) and loss of DNA repair capability (Rad51 foci). *In vivo* experiments confirmed the observations in preclinical settings of OC patient-derived xenografts representative of patients' disease.

These results represent a proof-of-principle that it is possible to improve and extend the therapeutic benefit of PARP inhibitors modulating cancer mitochondrial metabolism and set the basis to design novel therapeutic strategies.

Keywords: ovarian cancer, PARP inhibitors, DNA repair, mitochondrial metabolism

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T29

Lung disorders treatment: Investigating the impact of inhalable nanoformulations in a murine model of pulmonary fibrosis

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Pulmonary fibrosis is a chronic disorder involving lung parenchyma. It is characterized by the deposition of extracellular matrix leading to lung remodelling. Although this alteration can be due to different factors, it always impairs respiratory function and, in idiopathic forms, is fatal[1]. Macrophages play a key role in fibrosis acting as tissue sentinels and producing numerous pro-fibrotic factors. These cells localize in proximity to myofibroblasts, which are highly responsive to cytokines. Therefore, the inflammatory response enhances fibrogenesis[2]. The lack of efficient anti-fibrotic therapies prompted the scientific community to find alternative strategies to improve the lung tropism. Both intranasal administration and the use of nanocarriers might offer advantages in tackling this severe pathologic process[3]. My PhD project focused on intranasally-injected steroid-loaded nanoparticles (NPs) in healthy mice and in a murine model of pulmonary fibrosis. The biodistribution and pharmacokinetics of free and NP-linked dexamethasone were investigated both in vivo and ex vivo. NPs were able to penetrate the lungs rapidly and segregate inside lysosomes of pulmonary macrophages. The drug is released from NPs in lungs with a strong persistence in fibrotic conditions. These findings highlight the potential of using intranasal delivery of nanodrugs for targeted and efficient treatment of lung disorders.

Keywords: Nanomedicine - Pulmonary Fibrosis - Inflammation - Nanoparticles - Histopathology

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