THE One Health

TeCSBi PhD Meeting 2022

14th PhD Meeting

September 26-28, 2022

Certosa 1515, Avigliana (TO)

SCUOLA DI DOTTORATO
BICOCCA

Certosa 1515
Avigliana, Torino (Italy)
26-28 September 2022

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UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

SCUOLA DI DOTTORATO BICOCCA

Certosa 1515
Avigliana, Torino (Italy)
26-28 September 2022

THE one health: Technology, Human and Environment

DAY 1: Human

Monday 26th of September 2022

Webex link:
https://unimib.webex.com/unimib/j.php?MTID=m8413098ecf4200d40c9676f8cef20c28
PW: DAY1

08:30 – 08:45 *Opening:* **Prof. Paola Branduardi**, TeCSBi Coordinator

SESSION I

Chairs: Martina Arici, Lucia Morelli, Patricia Perez Schmidt

08:45 – 09:10 **Giulia Dellavedova** (*2nd year*) **So1**

"Overcoming PARP inhibitors drawbacks with a new generation PARP1 selective drug. Phase 2: Treatment with a next generation PARP1 selective inhibitor potentiates the efficacy of carboplatin in pre-clinical models of ovarian cancer"

09:10 – 9:35 **Jennifer Fernandez Alarcon** (*3rd year*) **So2**

"Evaluation of the role of surface functionalization of Glyco-NP on the distribution, excretion and crossing of biological barriers in mice"

9:35 – 10:00 **Laura Formenti** (*2nd year*) **So3**

"Oxidative metabolism and pharmacological response to PARP inhibition in ovarian cancer"

10:00 – 10:25 PhD STUDENT'S FLASH POSTER PRESENTATION: *1st year*

- *Filippo Testa* **Fo1**
- *Marina Meroni* **Fo2**
- *Roberta Pensotti* **Fo3**
- *Giulia de Simone* **Fo4**
- *Serena Petrella* **Fo5**

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10:25 – 11:10	POSTER SESSION I and COFFEE BREAK	
11:10 – 11:55	*Invited lecture*: ***Prof. Tarja Malm***, A.I.Virtanen Institute for Molecular Sciences, University of Eastern Finland, Finland **"Traffic related air pollution alter human microglial functions"**	
11:55 – 12:20	**Stefania Garbujo** *(3rd year)* "Design and development of cell-derived biomimetic nanoparticles as a potential tool for targeting cancer associated fibroblasts in the tumor microenvironment"	**S04**
12:20 – 12:45	**Elisa Perciballi** *(2nd year)* "Impact of SOD1 mutations on the metabolism of fibroblasts derived from Amyotrophic Lateral Sclerosis Patients"	**S05**
12:45 – 13:10	**Martina Arici** *(3rd year)* "SERCA2a protein purification to study interaction between SERCA2a-PLN complex and new istaroxime follow-on compounds"	**S06**
13:10 – 14:30	LUNCH BREAK	

SESSION II

Chairs: Jennifer Fernandez Alarcon, Stefania Garbujo

14:30 – 15:15	*Invited lecture*: ***Prof. Julian Quodbach***, Department of Pharmaceutics, Utrecht University, The Netherlands **"Can 3D printing solve poorly soluble drugs?"**	
15:15 – 15:40	**Lucia Morelli** *(3rd year)* "The study of polymeric nanoparticles stability after loading in oral dosage forms"	**S07**
15:40 – 16:05	**Annalisa Morelli** *(2nd year)* "Intranasal administration of dexamethasone-loaded nanoparticles improves lung tropism and reduces steroid off-target accumulation in healthy and in pulmonary fibrosis-affected mice"	**S08**
16:05 – 16:25	COFFEE BREAK	

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16:25 – 16:50 **Patricia Perez Schmidt** *(3rd year)* **S09**

"Synthesis of glycan-coated nanoparticles for the enhanced active targeting"

16:50 – 17:15 **Giacomo Ducci** *(2nd year)* **S10**

"Profiling metabolic and signalling phenotype of advanced cellular models of bladder cancer"

17:15 – 18:00 *Invited lecture*: ***Prof. Mauro Ferrari***, President and CEO of BrYet Pharma, USA

"My 30 Years of Failures: The Musical!"

In the evening: Social Dinner, music and dancing

END DAY 1

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DAY 2: Environment

Tuesday 27th of September 2022

Webex link:
https://unimib.webex.com/unimib/j.php?MTID=mdecb8cf786cc64540fce1de4da4e9d0d

PW: DAY2

SESSION III

Chairs: Greta Bianchi, Davide Panzeri

8:30 – 9:15	*Invited lecture*: ***Prof. William Martin***, Institute of Molecular Evolution, Heinrich-Heine-Universität Düsseldorf, Germany **"Energy at origins: The importance of the environment in early evolution"**
9:15 – 9:40	**Stefania Pagliari** *(2nd year)* **S11** "Optimization of Ultrasound-Assisted Extraction of naturally occurring glucosinolates from by-products of *Camelina sativa L.*"
9:40 – 10:05	**Letizia Maestroni** *(3rd year)* **S12** "Development of new combinations of synthetic biology approaches leads to significant improvements of microbial-based processes"
10:05 – 10:30	PhD STUDENT'S FLASH POSTER PRESENTATION: *1st year* • *Beatrice Negrini* **F06** • *Vittorio Senatore* **F07** • *Alessandro Marchetti* **F08** • *Sara Fumagalli* **F09** • *Mirko Zago* **F10**
10:30 – 11:15	POSTER SESSION II and COFFEE BREAK

11:15 – 11:40	**Marta Simonetti** *(2nd year)*	**S13**
	"Implementation of a bioeconomy business model in the textile industry"	
11:40 – 12:05	**Luca Mastella** *(3rd year)*	**S14**
	"Process and metabolic engineering for the optimization of vitamin B9 production in yeasts"	
12:05 – 12:30	**Emiliano Pioltelli** *(2nd year)*	**S15**
	"Impact of landscape anthropization on insect pollinators: a nutritional perspective"	
12:30 – 12:55	**Davide Panzeri** *(3rd year)*	**S16**
	"Deep insights of natural protease inhibitors in *Vigna unguiculata* (L.) Walp., from genetic diversity to biochemical characterisation"	
12:55 – 14:15	LUNCH BREAK	

SESSION IV

Chairs: Luca Mastella, Roberta Torinesi, Alex Pessina

14:15 – 15:00 *Invited lecture*: ***Prof. Pietro Tedesco***, Stazione Zoologica Anton Dohrn, Naples (ITA)

"Biosurfactants from extreme environments: from discovery to biotechnological applications

15:00 – 15:25	**Greta Bianchi** *(3rd year)*	**S17**
	"Charge patterning affects heterotypic protein-RNA interaction and phase separation behaviour of a model IDP"	
15:25 – 15:50	**Giulia Motta** *(2nd year)*	**S18**
	"Adverse outcome pathways-oriented toxicology in *in vitro* systems for implementing the safety-by-design of new nanomaterials: submerged and Air-Liquid-Interface exposure"	
15:50 – 16:10	COFFEE BREAK	

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16:10 – 16:35 **Roberta Torinesi** *(3rd year)* **S19**

"The role of ubiquitin- and Atg8-binding proteins in stress-induced ubiquitin-rich aggregates clearance "

16:35 – 17:00 **Vernay Thomas** *(2nd year)* **S20**

"Discovery of new potent antibiotic molecules from a rare actinomycete genus"

17:00 – 17:45 *Invited lecture:* ***Prof. Paola Vitale***, Department of Pharmacy-Drug Sciences, University of Bari "Aldo Moro", Interuniversity Consortium C.I.N.M.P.I.S., Italy

"Water and Deep Eutectic Solvents as promising reaction media toward new greener approaches for the preparation of fine chemicals and APIs"

In the evening: Social Dinner and free interaction

END DAY 2

DAY 3: Technology

Wednesday 28th of September 2022

Webex link:
https://unimib.webex.com/unimib/j.php?MTID=md76f19ca2ef286c5df0a29829073a159

PW: DAY3

SESSION V

Chairs: Letizia Maestroni, Lorenzo Rossi

08:30 – 08:55	**Carlo Rinaldi** *(3rd year)*	**S21**
	"Role of the Ku complex in the DNA damage response: end-tethering activity and regulation by Tel1/ATM kinase"	
08:55 – 9:20	**Federica Barbugian** *(2nd year)*	**S22**
	"3D bioprinted glioblastoma models for drug screening and to understand the role of ECM in malignancy"	
9:20 – 9:45	**Sara Bertuzzi** *(3rd year)*	**S23**
	"Molecular recognition of Glycans by lectins and beyond: a 3D view by using NMR"	

9:45 – 10:10 PhD STUDENT'S FLASH POSTER PRESENTATION: *1st year*

- *Elisa Dama* **F11**
- *Chiara Baioni* **F12**
- *Chiara Frigerio* **F13**
- *Pietro Butti* **F14**
- *Giuseppe Silvestri* **F15**

10:10 – 10:55 POSTER SESSION III and COFFEE BREAK

10:55 – 11:40 *Invited lecture*: ***Prof. Pablo I. Nikel-Mayer***, The Novo Nordisk Foundation Center for Biosustainability, Denmark

"Daring the limits of chemistry in living cells by engineering synthetic metabolism for biofluorination"

11:40 – 12:05 **Giulia Tomaino** *(2nd year)* **S24**

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

12:05 – 12:30 **Alex Pessina** *(3rd year)* **S25**

"Improved trehalose production using yeast"

12:30 – 12:55 **Michela Galli** *(2nd year)* **S26**

"Interplays between nucleases and helicases in the DNA damage response"

12:55 – 14:10 LUNCH BREAK

SESSION VI

Chairs: Carlo Rinaldi, Sara Bertuzzi

14:10 – 14:55 *Invited lecture*: ***Prof. Dina Zielinsky***, Inserm, Paris, France

"A future for digital data in nature's oldest storage device"

14:55 – 15:20 **Paolo Pizzul** *(2nd year)* **S27**

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

15:20 – 15:45 **Lorenzo Rossi** *(3rd year)* **S28**

"Chitosan: a versatile scaffold for self-assembled multivalent and multimodal nanomaterials"

15:45 – 16:30 *Invited lecture:* ***Prof. Dan Peer***, Laboratory of Precision NanoMedicine, Tel Aviv University, Israel

"RNA Therapeutics is Going Beyond the Liver: From Gene Silencing to Gene Editing"

16:30 – 17:00 *Awards and Greetings* - ***Prof. Paola Branduardi***

END DAY 3

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

14th PhD Meeting September 26-28, 2022

THE One Health

TeCSBi PhD Meeting 2022

Abstract Book

S01.Overcoming PARP inhibitors drawbacks with a new generation PARP1 selective drug

Phase 2: Treatment with a novel PARP1 selective inhibitor potentiates the efficacy of carboplatin in pre-clinical models of ovarian cancer

Giulia Dellavedova,[1,2] Alessandra Decio,[2] Laura Formenti,[1,2] Raffaella Giavazzi[2] and Maria Rosa Bani[2]

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PARP inhibitors currently used in clinic inhibit PARP1 and PARP2 causing adverse effects, which have limited their ability to be combined with chemotherapy[1]. Recently it has been suggested that inhibition of only PARP1 is required for antitumor activity, while the action on other PARPs may be responsible for the toxicity[2]. This hypothesis led to the development of next generation inhibitors with improved selectivity for PARP1[3], among them the potent inhibitor and trapper used in the study (**PARP1i**).

Here, the combination of **PARP1i** with carboplatin (CPT), a standard-of-care treatment for ovarian cancer, was investigated. Patient-derived ovarian cancer xenografts were implanted subcutis or orthotopically to assess treatment efficacy on tumour growth and metastatic dissemination.

In subcutaneously growing HOC106, CPT plus **PARP1i** treatment induced regression that persisted for at least 4 weeks after therapy withdrawal. Notably, in the poorly platinum-responsive HOC107, combination therapy stabilized tumour growth even at doses ineffective on their own.

Most importantly, the combination impaired metastatic dissemination and significantly prolonged the lifespan of HOC22 bearing mice (orthotopically implanted), even when CPT was administered at suboptimal doses, which were not effective as monotherapy.

Our results suggest that CPT and **PARP1i** combination drove superior efficacy compared to both monotherapies, thus being a possible option for ovarian cancer patients' benefit, and the advantages should be explored in clinical trials.

Keywords: Ovarian cancer xenografts, PARP1 inhibitor, Carboplatin

References

1. Martorana F., Da Silva L.A., Sessa C., Colombo I. (2022). *Cancers*, **14**, 953.
2. Ronson G.E., Piberger A.L., Higgs M.R., Olsen A.L. (2018). *Nature Communications*, **9**, 746.
3. Ngoi Y.L., Leo E., O'Connor M., Yap T.A. (2021). *The Cancer Journal*, **27**, 521-528.

S02. Evaluation of the role of surface functionalization of Glyco-NP on the distribution, excretion and crossing of biological barriers in mice

Jennifer Fernandez Alarcon [1]*, Mahmoud Soliman [2]*, Tanja Ludtke [3], Patricia Perez Schimdt [4], Alessandro Corbelli [1], Fabio Fiordaliso [1], Chiara Cordiglieri [5], Giovanni Sitia [6], Sergio Moya [3], Laura Polito [4]*, Marco P. Monopoli [2]* and Paolo Bigini [1]*

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Correlation among physicochemical properties of plasmonic nanoparticles (NPs) with their interactions in biological matrices is key for nanomedicine. One of the main issues is the uncertainty regarding nanomaterials behavior in humans body and other animals, due long-term accumulation. While biodistribution of NPs has been widely studied at organ levels[1], little is known about their accumulation in organs at sub-cellular level. Since the liver is the main filter organ for NPs, this work was aimed at evaluating hepatic accu-mulation, internalization and safety of NPs *in vivo* after a single administration. Herein, we compare the influence of shape, size, polymer coating and glycan functionalization on their fate in the hepatobiliary clearance using ICP-MS combined to advanced microscopy. Our data demonstrated that geometry and surface functionalization can dramatically change liver kinetics. We have further demonstrated the biocompatibility of gold NPs coated with monosaccharides and glycopeptides (glyco-NPs) and their ability to modify hepatic immunoresponse[2].

Overall, this study can be considered a platform to predict the subcellular disposition in organs and the impact of glyco-NPs on their biological fate as an engineering tool for the designing of nanomaterials towards clinical translation.

Keywords: gold nanoparticles, glycans, glycopeptide, kupffer cell, endothelial cell

References

1. Talamini, L., et al. (2017), Influence of Size and Shape on the Anatomical Distribution of Endotoxin-Free Gold Nanoparticles, *ACS Nano*, **11**(6): 5519-5529.
2. Reily, C., Stewart, T.J., Renfrow, M.B. et al. (2019), Glycosylation in health and disease. *Nat Rev Nephrol* **15**, 346–366.

S03. Oxidative metabolism and pharmacological response to PARP inhibition in ovarian cancer

Laura Formenti [1,2], Alessandra Decio [2], Giulia Dellavedova [1,2], Valentina Dematteis [2], Nicolò Panini [3], Raffaella Giavazzi [2], Maria Rosa Bani [2] and Carmen Ghilardi [2]
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[2] *Laboratory of Cancer Metastasis Therapeutics and* [3] *Laboratory of Anticancer Pharmacology, Department of Oncology, Mario Negri Institute of Pharmacological Research, IRCCS, Milan, Italy.*
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Ovarian cancer is the most fatal of all gynaecological cancers [1]. The clinical approach for its treatment was greatly transformed by the introduction of Poly(ADP-ribose) polymerase (PARP) inhibitors, which exploit homologous recombination (HR) deficiencies to specifically target cancer cells [2]. Recent observations suggested that there might be an interplay between HR status and cellular metabolism [3].

In this context, our aim is to investigate the reciprocal relationship between mitochondrial oxidative metabolism, HR functionality and sensitivity to PARP inhibition. To this end, we altered the oxidative metabolism of ovarian cancer cell lines.

Exploiting the knock down of a master regulator of mitochondrial functions, we observed that the alteration of oxidative metabolism was associated with an increased sensitivity to PARP inhibition. Using an inhibitor of the complex I of the electron transport chain, we evaluated the levels of co-factors, such as ATP and NADH, required for functional DNA repair machinery. No alteration of ATP was observed, while NADH levels increased upon OXPHOS inhibition.

These results suggest that the imbalance of redox equilibrium might play a role in the interplay between oxidative metabolism and HR functionality laying the ground for further studies to assess the connection between them and sensitivity to PARP inhibition.

Keywords

Mitochondrial oxidative metabolism, HR functionality, PARP inhibition, ovarian cancer

References

1. SEER Cancer Stat Facts: Ovarian Cancer. National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/statfacts/html/ovary.html.
2. William P. Tew, Christina Lacchetti, Annie Ellis, Kathleen Maxian, Susana Banerjee, Michael Bookman, Monica Brown Jones, Jung-Min Lee, Stéphanie Lheureux, Joyce F. Liu, Kathleen N. Moore, Carolyn Muller, Patricia Rodriguez, Christine Walsh, Shannon N. Westin, and Elise C. Kohn. (2020). Journal of Clinical Oncology 2020, 38(30), 3468-3493.
3. Lahiguera, Á., Hyroššová, P., Figueras, A., Garzón, D., Moreno, R., Soto-Cerrato, V., McNeish, I., Serra, V., Lazaro, C., Barretina, P., Brunet, J., Menéndez, J., Matias-Guiu, X., Vidal, A., Villanueva, A., Taylor-Harding, B., Tanaka, H., Orsulic, S., Junza, A., Yanes, O., … Viñals, F. (2020). EMBO molecular medicine, 12(6), e11217.

F02. Pharmacokinetic evaluation of trabectedin and pioglitazone combination in myxoid liposarcoma patient-derived xenografts

Marina Meroni[1,2], Cristina Matteo[2], Tommaso Ceruti[2], Lavinia Morosi[2], Massimo Zucchetti[2], Roberta Sanfilippo[3], Paolo Giovanni Casali[3], Maurizio D'Incalci[2] and Roberta Frapolli[2]

[1]*Department of Biotechnology and Biosciences, University of Milan-Bicocca, Milan (Italy),*
[2]*Laboratory of Cancer Pharmacology, Department of Oncology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, 20156 Milan, Italy.*
[3]*Medical Oncology Unit 2, Fondazione IRCCS Istituto Nazionale dei Tumori, 20133 Milan, Italy*.
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Myxoid liposarcoma (MLS) is characterized by an adipogenesis block that causes the accumulation of immature adipoblast with uncontrolled proliferation. Trabectedin reactivates adipogenesis but, although its good clinical efficacy in MLS patients, resistance occurs with no further effective therapies available[1]. We have recently demonstrated that the combination of trabectedin with the PPARγ-agonist pioglitazone restores adipocytic differentiation, overcoming trabectedin resistance[2]. This data led to the design of a clinical study in MLS patients.

To exclude unexpected interactions between pioglitazone and trabectedin, we evaluated their pharmacokinetics in ML017 (sensitive to trabectedin) and ML004 (resistant to trabectedin) MLS patient-derived xenografts. Mice received trabectedin and pioglitazone as single agents or in combination. Plasma, tumor, and liver were collected at different time points after single or repeated drug treatments.

We found that co-administration of trabectedin and pioglitazone did not induce major modifications in their plasma pharmacokinetics. Interestingly, the combination caused an increased trabectedin distribution in ML004 tumors, possibly due to changes in tumor tissue induced by pioglitazone that may contribute to the improved efficacy of the combination. Moreover, liver levels of both drugs were lower when administered in combination, and this aspect is particularly relevant, being the liver a target organ for trabectedin toxicity.

Keywords: myxoid liposarcoma, pharmacokinetics, trabectedin, pioglitazone

References

1. Di Giandomenico S, Frapolli R, Bello E, Uboldi S, Licandro SA, Marchini S, Beltrame L, Brich S, Mauro V, Tamborini E, Pilotti S, Casali PG, Grosso F, Sanfilippo R, Gronchi A, Mantovani R, Gatta R, Galmarini CM, Sousa-Faro JM, D'Incalci M. Oncogene. 2014 Oct 30;33(44):5201-10.
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F03. Nutraceutical approach to increase healthy aging using *Caenorhabditis elegans* as a model organism

Roberta Pensotti[1] and Maria Elena Regonesi[1]

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Humans are gradually moving towards an aging society. Aging is a process of gradual physiological decline and a risk factor for several pathologies[1]. Understanding the mechanisms underlying aging is fundamental to promote healthy aging, even if it is complicated by its multifactorial nature, in which environmental factors (e.g. nutrients) play an important role[2,3].

In this project, the main aging phenotypes (healthspan parameters) will be correlated with the major known nutrient-sensitive signalling pathways in *Caenorhabditis elegans*, a validated model for aging research. The first results showed a progressive decline of movement during *C. elegans* lifespan since the early adulthood. Otherwise, the heat stress resistance decreases only in old age; suggesting that the two parameters do not seem to be related. In future the other physiological phenotypes, i.e. reactive oxygen species accumulation, pumping rate and lipofuscin accumulation, will be assessed. Given the important impact of diet on healthy aging, the effect of the cinnamon bud extract on *C. elegans* lifespan and healthspan will be evaluated. The effective dose to assess cinnamon bud anti-aging properties was defined by heat stress test, pre-treating adult worms with a single dose for 48 hours. The next step will be the study of all the aging parameters in the presence of the extract.

Keywords: Aging, *Caenorhabditis elegans*, Nutrient, Healthspan

References

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F04. Set-up of mass spectrometry-based metabolomics methods for deciphering the metabolic states associated to macrophages morphology in colorectal liver metastasis

Giulia De Simone,[1,2] Roberta Pastorelli,[2] Laura Brunelli [2]

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Recent evidence indicates that the morphology of tumor-associated macrophages (TAMs) correlates with patient prognosis in colorectal liver metastasis (CLM). Specifically, small (S) and large (L) TAMs are associated with 5-year disease-free survival rates of 27.8% and 0.2%, respectively.[1]

This study is aimed at characterizing TAMs metabolic landscape to explore its correlation with morphology thus identifying better prognostic markers through mass spectrometry (MS)-based metabolomic approaches. Macrophages were FACS-sorted from surgically resected CLM tissues. We first evaluated the minimum number of cells required for metabolomic analysis testing 10'000, 20'000, 50'000 and 100'000 cells. Flow injection analysis–MS (FIA-MS) was used for the untargeted profiling, allowing the identification of more than 100'000 *m/z* without performing a chromatographic separation. We defined that 20'000 cells was the optimal number for our analysis. The method was applied to L-S TAMs derived from 14 CLM patients. We identified 1652 metabolites (78 lipids, 1574 non-lipids), using our *in-house* tool for FIA data pre-processing. Ten metabolites (e.g. aminoacids derivatives and lipid species) were statistically different between S and L-TAMs ($p<0.05$). Further, we will develop a target metabolomic strategy (LC-MS-MRM) to detect specifically metabolites mapping into central metabolic networks.

Keywords: Tumor associated macrophages, Metabolomics, Mass Spectrometry

References

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F05. Identification of new therapeutic strategies for mucinous ovarian carcinoma

Serena Petrella[1], Mirko Marabese[2], Marika Colombo[2], Marco Bolis[3], Laura di Rito[3], Luca Guarrera[3], Ilaria Craparotta[3], Maria Chiara Barbera[3], Nicolo Panini[4], Andrea Panfili[4], Valentina Sancisi[5], Massimo Broggini[2] and Giovanna Damia[1]

[1]Mario Negri Institute –Laboratory of Experimental Oncology, [2]Mario Negri Institute -Laboratory of Molecular Pharmacology; [3]Mario Negri Institute -Laboratory of Computational Oncology; [4]Mario Negri Institute -Laboratory of Antitumoral Pharmacology,[5]Laboratory of Translational Research, Azienda USL-IRCCS di Reggio Emilia

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Mucinous ovarian cancer (mEOC) is the rarest subtype of epithelial ovarian cancer; it is particularly aggressive and poorly responsive to chemotherapy[1]. Our lab has recently identified PLK1 (polo like kinase 1) as a druggable target in mEOC[2]. The present study aimed at identifying PLK1 synthetic lethal partners in mEOC lines using a CRISPR/Cas9 library approach. For constitutive Cas9 expression, three mucinous cell lines (MCAS, EFO27, TOV2414) were infected with the lentiCas9-Blast plasmid. Cas9 expression was confirmed by western blot analysis. PXPR_011 (lentivirus plasmid coding for both GFP and for GFP-guideRNA) was infected in Cas9 expressing cells to confirm Cas9 activity by detection of GFP negative cells (correlated with Cas9 activity) by flow cytometric analysis. We obtained 62,71% GFP- cells in MCAS Cas9, 70,47% GFP- in EFO27, and 55,92% GFP- in TOV2414. In the three cell lines the multiciplity of infection, and the dose of onvansertib to be used in combination with the infection of the CRISPR/Cas9 library were determined. All the experimental conditions for the screening experiment with the human CRISPR-Cas9 library (drug targets, kinases, phosphatases-deletion[3]) in combination with a sub-cytotoxic dose of onvansertib in the three different mEOC cell lines have been set up.

Keywords: Epithelial Ovarian Cancer, Mucinous Ovarian Cancer, CRISPR/Cas9, Onvansertib

References

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S04. Design and development of cell-derived biomimetic nanoparticles as a potential tool for targeting cancer associated fibroblasts in the tumor microenvironment

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The increased awareness of stromal contributions to cancer progression has led to a new cancer treatment paradigm: targeting the tumor stroma acquire primary role in the fight against cancer [1,2]. Among tumor microenvironment components (TME), cancer associated fibroblasts (CAFs) represent a pivotal player in TME modulation [3,4].
We propose an innovative therapeutic strategy consisting in reprogramming CAFs by means of gene therapy to kill cancer cells. Our approach relies on the use of cell membrane-derived biomimetic nanoparticles (CMNPs) for the delivery of TNF-related apoptosis-inducing ligand (TRAIL) encoding mRNA. CMNPs obtained from cancer cells are exploited for their inherent ability of homing the tumor site. The strategy selected for TRAIL mRNA loading onto CMNPs relies on the design of a core-shell system: lipid nanoparticles (LNPs) entrapping mRNA are first synthetized (core) and secondly coated with cancer cell membranes (shell). LNPs are manufactured by mixing an ethanol phase containing lipid components and an aqueous phase (mRNA molecules) using microfluidic approach which allows high mRNA encapsulation efficiency [5]. Afterward CMNPs coating was achieved by incubation with LNPs followed by sonication. The obtained population was characterized, and preliminary cell uptake studies were performed in CAFs by means of flow cytometry and confocal microscopy.

Keywords: biomimetic nanoparticles; tumor microenvironment; cancer associated fibroblasts; lipid nanoparticles; mRNA

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S05. Impact of SOD1 mutations on the metabolism of fibroblasts derived from Amyotrophic Lateral Sclerosis Patients

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of the upper and lower motor neurons (MNs). About 10% of patients have a family history; however, most patients seem to develop the sporadic form of the disease.
SOD1 (Cu/Zn superoxide dismutase-1) is the first studied gene among the ones related to ALS. Mutant SOD1 can adopt multiple misfolded conformations, lose the correct coordination of metal binding, decrease structural stability, and form aggregates[1]. For all these reasons, it is complicated to characterize the conformational alterations of the ALS-associated mutant SOD1, and how they relate to toxicity.
In this work, we performed a multilayered study on fibroblasts derived from two ALS patients, namely $SOD1^{L145F}$ and $SOD1^{S135N}$, carrying the p.L145F[2] and the p.S135N[3] missense variants, respectively. The patients showed diverse symptoms and disease progression, analogously to the bioinformatic analysis, which predicted a different effect of the two mutations on the protein structure. Interestingly, both mutations influenced the energy metabolisms. However, while the $SOD1^{L145}$F fibroblasts still relied more on oxidative phosphorylation, the $SOD1^{S135N}$ fibroblasts showed a metabolic shift toward glycolysis.
Our study suggests that SOD1 mutations might lead to alterations in the energy metabolism.

Keywords: ALS, SOD1 mutations, metabolism, seahorse

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S06. SERCA2a protein purification to study interaction between SERCA2a-PLN complex and new istaroxime follow-on compounds

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Agents that improve intracellular Ca^{2+} dynamics represent a favourable therapeutic approach for heart failure. Istaroxime is a promising agent combining Na^+/K^+ pump inhibition and SERCA2a stimulation [1]. Thanks to a joint lab in BTBS, we developed new istaroxime follow-on compounds able to improve cardiac performance in diseased animals by selectively SERCA2a stimulation [2, 3].

The aim of the project was to investigate the molecular mechanism of action of these new molecules, analysing their interaction with SERCA2a and/or its physiological inhibitor (PLN) through NMR studies. To do this, we started setting up the best protocol to purify SERCA2a; a synthetic PLN was used.

Preliminary NMR data showed that one of the new follow-on compounds was able to bind only the full length PLN_{1-52} like its parent compound istaroxime. Concerning SERCA2a purification, we started from pig cardiac microsomes (enriched vesicles of sarco-endoplasmic reticulum, ER), but we didn't reach a sufficient SERCA2a purity level. Thus, we moved to SERCA2a production in engineered hSERCA2a-YFP-His_8 *Saccharomyces cerevisiae* [4]. After 20h induction, the yeast effectively expressed SERCA2a and it mirrored the ER-protein localization pattern [5].

Overall, these data are preliminary to complete ligand-based NMR studies concerning SERCA2a/PLN complex interaction with istaroxime and its follow-on compounds.

Keywords: SERCA2a, Phospholamban, Istaroxime, NMR

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S07. The study of polymeric nanoparticles stability after loading in oral dosage forms

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Oral nanoparticles (NPs) have been selected as an alternative approach to improve the solubility and the stability of active ingredients in the gastrointestinal tract. The nanocarriers could reach both local and systemic drug targeting; allow a control release of encapsulated drugs, reducing the frequency of administration; and ameliorate patient's compliance[1,2]. After the development of a scale up protocol from single synthesis of NPs to continuous manufacturing production and their delivery in several oral devices systems, like pellets, minitablets or the newest "printlets; the following steps concerned the testing of their functionality verifying first of all the stability. As a consequence of the technological treatments to formulate NPs in oral dosage form, the size and polydispersity index are investigated and the stability are confirmed. These important results provide confidence on protection of encapsulated drug and lay the foundation for the following steps concerning the replacement of tracer drug, used for this preliminary data, with a biological molecule as ovalbumin

Keywords: oral nanoparticles, 3d printing, tablets, stability

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S08. Intranasal administration of dexamethasone-loaded nanoparticles improves lung tropism and reduces steroid off-target accumulation in healthy and in pulmonary fibrosis-affected mice

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Pulmonary fibrosis is a chronic disorder involving lung parenchyma. In patients, a paradoxical tissue repair response leads to irreversible scarring and lung remodelling due to an excessive deposition of extracellular matrix (ECM). This alteration impairs respiratory function and, in idiopathic forms is fatal[1]. Fibroblasts are key cells responsible for ECM deposition and upon different stimuli they can hyperproliferate at sites of injury and differentiate into myofibroblasts supporting the fibrotic process. These activated cells are highly responsive to growth factors/cytokines[2], indicating that inflammation and immune mechanisms contribute to fibrogenesis. Therapeutic approaches to arrest or at least reduce fibrosis still lack due to the difficulty of reaching the target. To this aim, it has been evaluated the targeting of intranasally-injected steroid-loaded nanoparticles (NPs)[3] 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 the lungs, avoiding blood circulation and off-target accumulation. These data suggest that this formulation can be a promising tool for the treatment of lung fibrosis and other autoimmune disorders.

Keywords: Nanomedicine – Lung Fibrosis - Inflammation - Nanoparticles - Histopathology

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S09. Synthesis of glycan coated nanoparticles for the enhanced active targeting

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Gold nanoparticles are a platform of interest with a broad range of applications, and they are emerging as a powerful tool in nanomedicine[1]. The surface chemistry based on the soft-soft interaction among gold and sulfur allows a reliable coating of the nanoparticles, paving the way to numbers of bio-applications. In particular, the multivalent presentation of carbohydrates can trigger a cluster effect which can overcome the low affinity of the individual ligands towards their receptors[2]. Moreover, the glycans surface modification can improve the gold nanoparticle circulation time in blood, tuning the formation of the "bio-corona"[3] and therefore preserving their active targeting[4]. In the last years, great efforts have been addressed to the synthesis and characterization of glyco-coated gold nanoparticles, in order to develop reliable and robust nanosystems which can be employed in many fields, from drug delivery to diagnosis[5].

Herein, we propose a new photo-induced one-pot synthesis based on a microfluidic approach to obtain a library of ultra-small glyco-gold nanoparticles (GAuNPs). GAuNPs were synthesized without the addition of template or reducing agents, affording fully characterized and size-controlled functionalized nanoparticles.

Keywords: gold nanoparticles, ultra-small, glyco-gold nanoparticles, glycans

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S10. Profiling metabolic and signalling phenotype of advanced cellular models of bladder cancer

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Bladder cancer (BC) is among the most common malignancies worldwide[1]. Progression of non-muscle invasive BC to invasive tumors and frequent recurrences reduce survival expectations[2,3]. Reprogramming of metabolism is a hallmark of cancer, representing attractive clinical targets exploitable in new therapeutic strategies[4]. Monolayer cultures poorly resemble the tumor structure, while three-dimensional cultures better recapitulate the *in vivo* architecture, cell-cell interactions, nutrients and oxygen gradients[5,6]. To understand the impact of 3D architecture on cellular metabolism in BC models, we first characterized morpho-functional features (proliferation rate, spheroid formation, migration, invasion) of six BC cell lines at different stages/grades grown as monolayers or spheroids. In collaboration with Temple University we are studying whether the EphA2/progranulin pathway, known for regulating motility, invasion and *in vivo* tumor formation[7], regulates spheroid formation. Energy metabolism studies using Seahorse technology show that cells grown in spheroids tend to increase glycolysis, although cell line-specific modulation is apparent. Functional metabolic data will be complemented with intra- and extracellular metabolomic profiling (in collaboration with CNR and University of Rome-Tor Vergata) and structured and integrated with transcriptomic-constrained analysis of mathematical models of metabolism. Understanding the mechanisms regulating metabolic changes of BC cells might contribute to identify novel targets and therapies for bladder cancer.

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

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S11. Optimization of Ultrasound-Assisted Extraction of naturally occurring glucosinolates from by-products of *Camelina sativa L.*

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Food supply chain produces huge amount of wastes, with high cost of disposal and environment impact [1] however, these agro-food wastes should be considered a by-product as source of bioactive compounds. Glucosinolates (GLSs) are a class of secondary metabolites widespread in particularly in the Brassicaceae family, with beneficial effects on human health [2-3]. *Camelina sativa*, cultivated for its seed rich in fatty acid [4], is one of the plants containing GLSs. Upon pressing, the seed pressed cake (PC) is a by-product that could be used as a source of bioactive compounds. Thus, the aim of this work is to develop a green extraction method to maximize the extraction yield of bioactive compounds, reducing the time, costs, and environmental impact of the process.
Initially, PC were characterized using ultra-pressure liquid chromatography coupled with a high-resolution mass spectrometry detector, identifying several GLSs. Then an extraction method was developed by ultrasound assisted technology using green solvents (water and ethanol). All the extraction parameters were optimized by experimental design and the GLSs were purified and concentrated by solid phase extraction. Finally, the extract was tested on intestinal cancer cells, showing a promising cytotoxic activity.
Future prospects will be to develop of automatized pressurized liquid-extraction method to improve extraction efficiency and reduce the ethanol used.

Keywords: *Camelina sativa* L., food by-products, glucosinolates derivatives, ultrasound assisted extraction, experimental design optimization, human colorectal cancer.

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S12. Development of new combinations of synthetic biology approaches leads to significant improvements of microbial-based processes

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Many microbial pathways are known to lead to the production of industrially relevant molecules. Indeed, microbial cells show several advantages to produce fine and bulk chemicals compared to conventional chemical synthesis, especially for compounds with complex structures. The yeast *Saccharomyces cerevisiae* is a platform of election as a chassis for these purposes[1].

The development of cell factories requires introduction of heterologous genes and rewiring of endogenous metabolism, with the aim of maximizing yield, production and productivity of the desired product[2,3]. The difficulty in obtaining ready-to-market products in a robust way and in short time still represents a limit of biotechnological productions: cell factories design and construction is still not standardized enough and depends on the product.

Starting from literature[4], we designed an original combination of standardized, modular and re-usable tools meant to make the process of microorganisms engineering rationally predictable. We will show with case studies how the developed synthetic biology toolkits lead to an acceleration in the construction of chassis and this big improvement leaves the space to better characterize the heterologous biosynthetic pathway in the final host, to analyse the metabolic flux inside the chassis and, overall, to improve yields, titers and productivities of final strains.

Keywords: Synthetic biology, pathway engineering, metabolic engineering, *Saccharomyces cerevisiae*

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F06. Safe and Sustainable nano-enabled antimicrobials to reduce the presence of contaminants of emerging concern (biotic and abiotic) in the aquatic environments

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Contaminants of emerging concern (CECs), such as antibiotics and antimicrobial resistant (AMR) bacteria in aquatic ecosystems, pose a serious threat to the environmental and human health. The increasingly widespread use of antibiotics in various fields facilitates the emergence of AMR bacteria, with which they largely end up in the environment and potentially affect human health through contaminated water and the food chain [1]. Nanomaterials (NMs) emerged as novel antimicrobial agents with proven efficacy against AMR bacteria [2]. However, uncertainties persist about their safety and environmental sustainability, as evidenced by toxicological and Life Cycle Assessment (LCA) studies [3,4]. Several European projects are engaged in the development of novel nano-enabled products aimed at addressing the problem of CECs by adopting a safe- and sustainable-by design strategy. In this framework, this PhD project is evaluating the safety of novel metal-based antimicrobial NMs. During the first year, sonochemically synthesized CuO nanoparticles (from Bar-Ilan University) have been characterized and their biological effects evaluated using zebrafish (*D. rerio*). The Fish Embryo acute Toxicity test (OECD, 2013) is being performed to define concentration-response curves for lethal and malformation effects. Morphometric and molecular analyses are being developed to decipher the mechanistic aspect of the CuO-induced toxicity. A comparative approach will be used to characterize the toxic profile of other nano-antimicrobials (e.g., AgNPs). The nanotoxicology results will be sided by LCA analyses, in order to contribute to a safe and sustainable development of new nano-biocidals.

Keywords: Nanoparticles; nanotoxicology; in vivo toxicity test; life cycle assessment; safe and sustainable by design.

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F07. REPLAY – Sustainable upcycling PET towards platform chemicals

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The use of plastics is unavoidable in our society due to their unique mechanical properties and low cost compared to other materials. Regrettably, the biodegradation of traditional plastic is extremely slow and occurs only rarely and only in specific niches: most of the plastic waste is burnt for heat or landfilled, representing a loss of resources and creating a cascade of environmental issues.
The project REPLAY aims at the upcycling of PET from post-consumer plastic waste through sustainable depolymerization and fermentation, towards the production of platform chemicals. Research teams from UNINA and UNIBA will develop sustainable strategies for the (bio)chemical hydrolysis of PET in its monomers terephthalic acid (TPA) and ethylene glycol (EG). My work will focus on the implementation of the use of systems and synthetic biology, and (bio)process engineering to enable yeasts (*Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts, among which *Zygosaccharomyces parabailii*) to convert TPA and EG into different organic acids (e.g. protocatechuic acid, *cis,cis*-muconic acid, and 3-carboxy-*cis,cis*-muconic acid).

Keywords:
Polyethylene terephthalate, *Saccharomyces cerevisiae*, *Zygosaccharomyces parabailii*, Cell factory, Synthetic biology

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F08. Antarctic glycosyl hydrolases for marine polysaccharides degradation: from discovery to characterization

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As temperature is considered a driving force in evolutionary pathways, one of the most adopted strategies by psychrophilic organisms to thrive in cold environments is the expression of cold-active enzymes. Usually, cold-active enzymes show high activities at low temperatures coupled with high structural flexibility and thermolability. These features make cold active enzymes attractive in biotechnological applications[1].

Marine polysaccharides constitute a widely available biomass, but this resource is still little considered and exploited[2]. Among polysaccharides degrading enzymes, glycoside hydrolases catalyse the breakdown of glycosidic bonds present in polysaccharides and oligosaccharides.

Here, we report the identification and characterization of a glycoside hydrolase belonging to family 3, namely M-βGl3. This enzyme has been identified in the genome of *Marinomonas* sp. ef1, an Antarctic marine bacterium. M-βGl3 is active at 5°C and displays a T_{opt} of 50°C and high glucose tolerance. Interestingly, M-βGl3 is active over a wide range of substrate, from glucose to xylose. The future aims of the project are to investigate the cold adaptation strategy of MβGl3 and explore its role in marine polysaccharide degradation.

Keywords: cold-active enzyme; glycoside hydrolase; marine polysaccharides; cold adaptation

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F09. SKIOME project: lead skin microbiome research towards interdisciplinary approaches

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Microbial communities play a significant role in human health, and skin microbiota is no exception. Many skin diseases are driven by skin microbiota, usually due to common commensal microorganisms' imbalance, named dysbiosis[1]. However, the skin ecosystem complexity hindered the characterization of representative skin microbiota profiles.

To capture the overall microbiome diversity and understand these complex processes, multiple datasets must be integrated for meta-analysis, and a variety of *in silico* analyses must be conducted[2]. Therefore, SKIOME project combines a data-driven approach with wet lab strategies aiming to identify microbial biodiversity, characterize microbial networks, and determine microorganisms' functional properties.

We are developing a bioinformatic strategy able to facilitate and automatize datasets integration, which represents the first meta-analysis step. As a first case study, we selected healthy face samples present in SKIOME collection[3], a curated collection of 16S rRNA amplicon-sequencing skin metagenomics datasets of the last decade. Data were integrated, harmonized and re-analyzed. From our preliminary results, we evidenced differences between diverse face sites, and we documented the biases introduced by the lack of standardization both in experimental and bioinformatic protocols.

Our work aims to establish a baseline in healthy skin microbiota, potentially contributing in cosmetics and medicine.

Keywords: skin microbiome, metagenomics, data integration

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F10. Novel biotechnological route for the production of low molecular weight (LMW) esters

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Sustainable synthesis of bulk chemicals, like low molecular weight (LMW) esters, is one of the most demanding challenges facing the chemical industry, in particular due to the low market price[1] of the petrochemical counterparts. In fact, in industrial practice LMW esters, like ethyl acetate, are still produced mainly through unsustainable chemical processes, with energy intensive procedures and starting from fossil-based feedstocks[1,2]. Nevertheless, extensive research has been done in the last 30 years to find competitive and viable ways of producing LMW esters via microbial fermentation or using specific enzymes as biocatalysts. To this end, in this project we are exploring the possibility and the feasibility of the production of LMW esters, like methyl formate, ethyl acetate and ethyl lactate, starting from renewable resources with a view to circular economy and carbon neutral balance[1]. Esters formation and quantification was initially carried out with use of a colorimetric assay[3] and will proceed by testing different enzymes, biological catalysts and reaction conditions to assess the most promising processes and productions, to further develop techno economic evaluations.

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

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S13. Implementation of a bioeconomy business model in the textile industry.

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The Textile Industry is one of the most environmentally polluting fields due to its linear business model [1]. One of the main environmental impacts results from the overproduction of pre-consumer and post-consumer waste. Worldwide, only around 1% of textile waste is currently recycled, and of this amount, in the EU, 5.8 million ton/year end up in landfills [2].
The aim of the project is to develop a circular economy model that allows to pursue the "zero waste" principle, utilising side streams and building blocks released from the hydrolysis of wastes to produce new molecules of interest within the production chain by means of microbial biotransformation processes, enhancing industrial synergy and bioeconomy [3].
To create a sustainable business model, the first goal of the project was to investigate an approach for the valorisation of waste from the textile manufacturing production sites. Here we describe an optimized protocol for the hydrolysis of pre consumer textile waste to obtain glucose. The strategy comprises a combination of treatments, exploiting a second residual NaOH-rich effluent from the finishing process waste and enzymatic hydrolysis, reaching out glucose liberation yields up to 85%.
The second goal was to implement the equipment required to carry out fermentation processes within the company, opening to the bioeconomy business model based on the exploitation of glucose recovered, applying the biorefinery framework.

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

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S14. Process and metabolic engineering for the optimization of Vitamin B9 production in yeasts

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Folates (vitamin B_9) are humans' essential micronutrients that function as cofactors in one-carbon transfer reactions involved in the synthesis of nucleotides and amino acids. Folates deficiency is associated with important diseases, making their supplementation through diet important [1]. All the vitamins B_9 commercially available are chemically synthetized in folic acid form, which does not maximize their biological effects; the production of natural folates by microbial fermentation therefore appears as a sustainable and preferable alternative [2]. *Saccharomyces cerevisiae* is a natural producer of folates and it has been engineered in the past for increasing the endogenous production, obtaining the best results by overexpressing the gene *FOL2* [3], encoding for a GTP-cyclohydrolase, responsible for the biosynthesis of one of the moiety of this complex molecule. However, the studies performed so far did not systematically combine the different manipulations of the genes present in the folate biosynthesis pathway with that one of the shikimate pathway.
Here we focused our engineering on unlocking the production of the building blocks of folate, 4-Aminobenzoic acid (pABA) and dihydropteridine, overexpressing genes related to those pathways. Overall, combining the simultaneous overexpression of 6 or 8 different genes and changing the integration locus thanks to the Easy-MISE tool kit (developed in our lab, manuscript in preparation), we developed the strains AFS_n and AFS_nXP. The folate production of these strains exceed that one of the *FOL2* overexpressing strain 46,6 ± 5,04 µg/L, reaching a production of 81,2 ± 6,95 µg/L and 76,7 ± 4,37 µg/L respectively.

Keywords: *S. cerevisiae*, Molecular biology, Vitamin B_9

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S15. IMPACT OF LANDSCAPE ANTHROPIZATION ON INSECT POLLINATORS: A NUTRITIONAL PERSPECTIVE

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Urbanization is dramatically modifying the environment occupied by insect pollinators mainly through the loss and fragmentation of green areas [1]. These disturbances, along with other anthropogenic pressures, impose constrictions to the foraging activity of pollinators and can additionally determine variations in the nutritional profile of floral resources [2]. In this context, unravelling the impact of landscape anthropization on the diet of pollinators is a critical step for their conservation [3] but a huge knowledge gap persists. In this study we investigated, for the very first time, the nutritional features of *Bombus terrestris* (Hymenoptera:Apidae) diet and the chemical composition of floral resources (i.e., nectar and pollen) in the metropolitan area of Milan through a multidisciplinary approach. The aim is to detect variations in the macronutrient and micronutrient composition in response to landscape anthropization by using different chemical analytics pipelines. We observed an overall worsening of the bumblebees' diet quality (i.e., decrease in protein content and in the protein:lipid ratio) together with a decrease in the antioxidant capacity of the nutritional landscape in more urbanized environments. Our results could be of key interest to provide valuable guidelines for urban planning and design aimed at ensuring the strengthening of pollinators conservation measures.

Keywords: Insect pollinators, Phytochemistry, Nutritional ecology

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S16. Deep insights of natural protease inhibitors in *Vigna unguiculata* (L.) Walp., from genetic diversity to biochemical characterisation

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Bowman-Birk protease inhibitors (BBIs) are a group of small proteins widely found in Fabaceae familys. Their biological purpose is to defend the plant from insects by inhibiting trypsin and chymotrypsin. In addition, BBIs are known to have promising nutraceutical bioactivities, in particular, they are able to act as chemopreventive agents[1,2]. However, despite these interesting properties there is still little knowledge in *Vigna unguiculata*. We evaluated the natural genetic biodiversity of two BBI genes encoding the two major BBI isoinhibitors, a trypsin-trypsin BBI (TT) and a trypsin-chymotrypsin BBI (TC). The TT screening was concluded by analysing around 200 *Vigna unguiculata* species and subspecies accessions coming majorly from Africa, while TC started on the same sample set. Network and phylogenetic analyses have elucidated the presence of a “core” TT haplotype (A2) with many connections with other haplotypes suggesting to be the one of the firstly appeared. In order to understand better the inhibition mechanism, we submitted the found deduced isoforms to computational analyses and some amino acid changes can effectively modify the BBI-target interaction. Parallelly, we extracted and purified BBIs directly from seeds to understand the profile naturally present. We then successfully identified and characterised the TC BBI and partially purified 6 TT BBI isoforms.

Keywords: Bowman-Birk inhibitors, Genetic Diversity, Phylogenetic Anlyses, Computational Evaluations, Protein Characterisation**.**

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S17. Charge patterning affects heterotypic protein-RNA interaction and phase separation behaviour of a model IDP

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Liquid-liquid phase separation (LLPS) occurs in polymeric systems under supersaturation conditions, resulting in a demixing of solute molecules from solvent ones[1]. In the overcrowded cellular environment, LLPS involves proteins and nucleic acids, producing the so-called “membraneless organelles”[2]. Intrinsically disordered proteins (IDPs) and regions (IDRs) display a unique propensity to undergo phase separation, due to their multivalency and amino acid composition[3]. Electrostatic forces were proved to have a crucial role in IDR-mediated liquid demixing. Both net charge and charge patterning – *i.e.* the linear distribution of charged residues – modulate IDR propensity to phase separate[4-6]. Nevertheless, a comprehensive understanding of such a correlation is still lacking. Therefore, this phenomenon was investigated on the N-terminal region of human topoisomerase I. Once the LLPS was assessed for the wild-type protein, two scrambled mutants were designed differing from it in the patterning of oppositely charged residues. A comparison of their LLPS propensities with wild-type protein was performed employing two different stimuli, namely exposure to RNA and drastic pH changes. It emerges that increasing charge segregation promotes the LLPS behavior of wild-type protein, but also confers anomalous characteristics to the coacervates. Overall, the results obtained help define the chemical-physical characteristics of the condensates and the biological significance of the statistically prevalent charge distribution among natural IDPs/IDRs.

Keywords: intrinsically disordered proteins; liquid-liquid phase separation; electrostatics; RNA; topoisomerase I.

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S18. Adverse outcome pathways-oriented toxicology in *in vitro* systems for implementing the safety-by-design of new nanomaterials: submerged and Air-Liquid-Interface exposure

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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.

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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S19. The role of ubiquitin- and Atg8-binding proteins in stress-induced ubiquitin-rich aggregates clearance

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The formation of protein aggregates is a conserved process engaged by eukaryotic cells in response to external stimuli that alter the physiological cellular environment. The yeast *Saccharomyces cerevisiae* is widely used for the research on protein aggregates, being recognized as an ideal system to investigate fundamental mechanisms[1]. In previous studies, we demonstrated the formation of ubiquitin-rich protein aggregates in *S. cerevisiae* following the exposure to ethanol stress and under nitrogen starvation conditions. Whereas the nitrogen starvation condition induced protein aggregates that are cleared by aggrephagy, the selective autophagy of protein aggregates, it is not known what mechanisms are involved in the clearance of protein aggregates under ethanol stress. The yeast protein Cue5 is an aggrephagy receptor, which possesses the ubiquitin-binding domain (CUE) and a Atg8-interacting motif (AIM)[2]. In addition to the ubiquitinated protein aggregates, it is also recruited to the ubiquitinated lipid droplets, following them to the vacuole for degradation by lipophagy, the selective autophagy of lipid droplets. Cue5 represents an appealing candidate for the aggrephagy receptor under ethanol stress. However, since Cue5 acts only as a "passenger" of lipophagy[3], other ubiquitin-binding autophagic receptors may also be involved. Hence, the aim of this study is to understand what receptors direct ubiquitin-rich aggregates degradation under ethanol stress.

Keywords: yeast, protein aggregates, autophagy, aggrephagy, Cue5

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S20. DISCOVERY OF NEW POTENTIAL ANTIBIOTIC MOLECULES FROM A RARE ACTINOMYCETE 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, 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 extracts. Combining antibiotic activity screening and metabolomic tools (MZMine2, GNPS, CompoundDiscoverer, …) to analyze this library, we are identifying some potentially promising features. We are also exploring the genomic biosynthetic potential of this genus thanks to full genome sequencing and Biosynthetic Gene Cluster identification through antiSMASH.

Keywords: drug discovery – actinomycetes – paired omics – antibiotics

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S21. Role of the Ku complex in the DNA damage response: end-tethering activity and regulation by Tel1/ATM kinase

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The repair of DNA double-strand breaks (DSBs) is essential to ensure genomic stability and to avoid cell death[1]. Non-homologous end joining (NHEJ) is one of the main mechanisms repairing DSBs and it is induced by the binding to DSBs of the Ku70-Ku80 heterodimer[2,3]. To better understand the role of the Ku complex in DSB repair, we searched for *ku70* mutations that suppress the sensitivity to DNA damaging agents of cells lacking Sae2, a protein involved in early steps of DSB processing. The *ku70-C85Y* allele causes an increase of Ku70 association close to the DSB ends and a suppression of the end-tethering defect of *sae2*Δ cells, suggesting a role of the Ku complex to keep the DNA ends close to each other. Moreover, Ku persistence at DSBs and its tethering activity are enhanced when histone removal around DSBs is impaired by eliminating either nucleosome remodelers or the kinase activity of Tel1, a fundamental protein involved in the DNA damage response. These findings show that Tel1 antagonizes the Ku tethering function by promoting nucleosome removal and Ku sliding inwards, suggesting that this Tel1 function can be important to regulate the mode by which DSBs are repaired.

Keywords: double-strand break, Ku70-Ku80, Tel1, end-tethering

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S22. 3D BIOPRINTED GLIOBLASTOMA MODELS FOR DRUG SCREENING AND TO UNDERSTAND THE ROLE OF ECM IN MALIGNANCY

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The extracellular matrix (ECM) is a dynamic microenvironment in which proteins, glycoproteins and GAGs favor tumor progression and invasiveness and in particular hyaluronic acid (HA) plays a key role in processes associated to major invasiveness and therapeutic failure[1].

The generation of glycoconjugate biopolymers to mimic ECM composition is a fascinating way to obtain tailorable *in vitro* systems able to replicate the physical and biochemical features of cell microenvironment[2,3].

In this work, HA has been crosslinked with different ECM proteins taking advantage of linkers with different lengths and branching. The final goal of the project is to understand and to test the effect of biochemical and physical behavior in GBM microenvironment.

The selected formulations were then tested with three different cell lines to obtain an in vitro 3D bioprinted GBM model suitable for high performance predictive screening and studying tumor microenvironment. Furthermore, by applying a flow rate, differences from static and dynamic condition were exploited. The lack of vascularization remains a major limitation. To recreate a complex and physiologically relevant vascularized tissue, we also fabricated a vascularized tissue model by combining template leaching additive manufacturing with hydrogels. The tissue model was then characterized for the successful vascularization of the channels built by template leaching, and the functionality of the surrounding tissue [4].

Keywords: Bioprinting, Regenerative medicine, Glioblastoma

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F11. A microRNA-based liquid biopsy signature for the early detection of lung cancer: a multi-centric study with a multi-platform workflow

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Lung cancer (LC) is the leading cause of cancer death[1]. Development of effective LC screening, combining LDCT with risk-prediction models based on biomarkers, are a clear unmet-clinical-need to augment early detection and reduce mortality. The lack of large validation studies as well as of closer-to-the-clinic optimized biomarkers limit the successful translation of risk-prediction models for LC early detection[2]. We therefore designed a multi-centric study including 389 lung tumors and 531 controls so far, from 7 European and US centers, to identify a microRNA-based signature easy detectable with PCR-profiling from liquid biopsy. To enhance validity across technology, we set-up a miRNA-profiling workflow by microarray, qRT-PCR, and dPCR. An *in-silico* analysis of 3 published datasets allowed the identification of 45 circulating-miRNAs differentially regulated between 150 tumors and 136 controls. We confirmed the reliable detection of these biomarkers in a cohort of 54 tumors and 54 controls, and we reduced our signature to 9 miRNAs by applying feature-selection methods in a larger plasma cohort (152 tumors, 261 controls) profiled by qRT-PCR. We are now testing our risk-model with dPCR to simplify its usage, and working with plasma from patients with lung nodules ≤30 mm which are difficult to manage by the sole CT imaging[3].

Keywords: cancer risk assessment; liquid biopsy; miRNAs; early lung cancer

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F12. Synthesis and spatio-temporal imaging of nanoparticles in cultured cells by Ultrafast Electron Microscopy for tumor theranostics

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The SMART-electron project aims at developing an innovative technological platform for designing, realizing and operating all-optical rapidly-programmable phase masks for electrons. A new paradigm where properly synthesized ultrafast electromagnetic fields will be used for engineering the phase space of a free-electron wave function will be introduced [1]. Using a Quantum Cathodoluminescence scheme based on an enhanced light emission when materials are interrogated with structured free-electron waves, we aim to investigate unique spatio-temporal localization of nanoparticles in cells for drug delivery and nanomedicine applications.

State-of-the-art electron microscopy analysis will be coupled with several other experimental approaches, some of which already established for the study of nanoparticle uptake (i.e. flow cytometry, confocal microscopy, hyperspectral darkfield microscopy), together with newly emerging techniques such as high-resolution fluorescence microscopies (SMLM) and correlative light/electron microscopy (CLEM), to evaluate the nanoparticle-cell interaction in terms of internalization, escape capabilities from the endo-lysosomal compartment and subsequent intra-cellular trafficking [2].

In the first year of my PhD, a method for the *in-situ* synthesis of gold nanoparticles inside a liposomal bilayer, through gallic acid mediated reduction of $HAuCl_4$ has been optimized. The so called Lipo-Gold have been characterized by means of standard DLS, NTA and TEM/SEM analysis and preliminary cytotoxicity and cellular uptake experiments have been performed on HeLa cancer cells.

Keywords: Lipid nanoparticles; Gold nanoparticles; Nanoparticles internalization; Endosomal escape; Ultrafast electron microscopy

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F13. INTERCONNECTIONS BETWEEN Fe-S CLUSTER BIOGENESIS AND GENOME STABILITY

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Iron-sulfur (Fe-S) clusters are small inorganic protein cofactors that function as electron carriers in redox reactions and devices for stabilization of protein domains. Maturation of cytosolic and nuclear Fe-S proteins requires the cooperation of the mitochondrial cluster assembly (ISC) and the cytosolic protein assembly (CIA) systems [1]. CIA proteins can form different sub-complexes which promote the insertion of Fe-S clusters into specific apoproteins. Some CIA proteins assist the maturation of factors involved in DNA replication, DNA repair, transcription and chromosome segregation, thus suggesting a link between Fe-S cluster biogenesis and genome stability [2]. The CIA system is conserved from yeast to humans, where mutations in Fe-S enzymes regulating DNA metabolism have been linked to several diseases and types of cancer [3].

In a genomic screening in *Saccharomyces cerevisiae* we identified mutations in CIA genes that increase resistance to the DNA damaging agents camptothecin and phleomycin and suppress the DNA damage sensitivity of cells lacking specific DNA damage factors. We are now exploring the molecular mechanisms underlying this suppression to better figure out how the CIA system can modulate the cellular response to DNA damage and ensure genome stability.

Keywords: *Saccharomyces cerevisiae*, iron-sulfur cluster, genome stability

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F15. Calculations of accurate flavodoxin's relative redox potentials using a non-equilibrium TI approach

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The flavoprotein cofactors mediate electron transfer (eT) in several biological processes and engineering the redox potentials of flavoproteins is of practical interest: current and future biotechnology applications include biosensors, biocatalysis, bioremediation, and bioelectronics[1]. Most of the information regarding the potential reduction (PR) stabilization of flavin by apoprotein comes from traditional site-directed mutagenesis and functional assays: the computational approach would allow for a large number of in silico mutagenesis experiments, accelerating the discovery of residues critical for PR modulation in terms of time and greatly reducing the experimental and economic effort to obtain some insights regarding this mechanism. To this end, the Non-Equilibrium Thermodynamic Integration[2] (NE-TI) method was applied on Clostridium Beijerinckii flavodoxin's structures derived from Molecular Dynamics (DM) simulations to obtain the free energy changes (and hence PRs) associated with individual reduction reactions in which flavin is involved. Its application in this work, therefore, is a real novelty in the field of PR calculation and may prove to be a useful tool for quantitative in silico screening of the effect of mutations on reduction potential.

Keywords: Redox Potential, Flavoprotein, Molecular Dynamics, Thermodynamic Integration

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S24. Recombinant vault nanoparticle: a potential tool for the targeted delivery of 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 *P. pastoris* has been used to constitutively express human recombinant vaults, subsequently purified by size exclusion chromatography, following a simple procedure here optimized. Purified recombinant vaults display the same morphology, size, and biological properties than their natural counterpart, as shown by transmission electron microscopy, dynamic light scattering analysis and endocytic studies. The same technologies have been used to produce and characterize a fusion protein between MVP and Z-peptide, 33aa derived from protein A that selectively binds antibody constant portion. Fluorometric analysis, on Vault-Z particle conjugated with a labelled antibody, proved its capacity to enable antibody-direct binding, a crucial aspect to promote antibody-mediated vault targeting to specific receptors at the cells' surface.
Small interfering RNAs have promising pharmacological potential but its realization depends on their ability to reach their targets in vivo[2]. We here attempt to load vaults with siRNAs targeting *LADON*, a lncRNA transcript recently found to promote invasive behavior in melanoma cells[3]. Vault complexes carrying anti-*LADON* siRNAs will then be used to target melanoma cells, where their ability to decrease *LADON* expression and *LADON*-dependent effects will be tested.

Keywords: vault protein, nanocarrier, Pichia pastoris, siRNA delivery, melanoma

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S25. Improved trehalose production using yeast

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Trehalose is a natural occurring sugar with growing interest not only in the food industry but also in cosmetic and pharmaceutical applications[1–3]. The oligosaccharides enzymatic conversion began the mass production process but with major issues: the use of several enzymes, the high by-product accumulation and complex purification steps from a mixture of various sugars[4]. The global Trehalose market was valued at 350.27 million USD in 2022, growing at a CAGR of 5,34% during 2020-2025[5].

In this study, an alternative approach exploiting the natural trehalose synthesis of *Saccharomyces cerevisiae* was developed, increasing fluxes toward trehalose secretion. Applying strain improvements and a fed-batch processes a high-purity trehalose can be easily obtained from the fermentation broth and with different feeding strategies we boost the titres to the highest known from a *S. cerevisiae* culture reported to date[2]. Furthermore, a high cell density continuous reactor was successfully applied suggesting possible extension of production phase as well as further improvements in yield and productivity. In addition, we are working on identifying a trehalose transporter that could increase the excreted fraction pushing the process to the limits.

Keywords: *S. cerevisiae*, enhanced metabolism, trehalose, fed-batch culture, continuous high-density culture.

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S26. Interplays between nucleases and helicases in the DNA damage response

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DNA double strand breaks (DSBs) are the most cytotoxic lesions that continuously threat our genome and could lead to genome instability. They are sensed by the protein kinase ATM (Tel1 in yeast), which orchestrates a complex genetic network, thus leading to efficient DSB repair [1]. DSBs can be repaired by two major cellular pathways: nonhomologous end joining (NHEJ), which provides the direct ligation of DNA ends, and homologous recombination (HR), which is initiated by the nucleolytic degradation of the 5' DSB ends in a process called resection [2]. Resection is a two-step process in which CtIP/Sae2 activates Mre11 endonuclease to initiate DNA resection and create a substrate for the nucleases Exo1 and Dna2, whose action generates long single-stranded DNA tails. To better define the functions and regulation of nucleases in response to DNA damage, as well as their interplays with ATM/Tel1, we used the budding yeast *Saccharomyces cerevisiae* and performed a genomic screening searching for extragenic suppressors of the hypersensitivity to DNA damaging agents of cells lacking both Tel1 and Exo1. We identified mutations in nucleases/helicases implicated in resection. Understanding the molecular mechanism underlying these genetic interactions could contribute to unravel novel interplays between Tel1 and Exo1 in DNA damage response.

Keywords:

Saccharomyces cerevisiae, DDR, Exo1, nucleases

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S27. 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 generated Rif2 mutations in the N-terminal domain[3] that can enhance the Rad50 and Rif2 interaction. We identified a *rif2* allele carrying a single amino acid change that exacerbates the sensitivity to DNA damaging agents of *sae2Δ* cells. We are characterising this allele to understand the molecular mechanisms underlying the increased DNA damage sensitivity that could provide new insights into the role of Rif2 in DNA damage response.

Keywords: *Saccharomyces cerevisiae*, DNA damage, MRX, Rif2, Sae2

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S28. Chitosan: a versatile scaffold for self-assembled multivalent and multimodal nanomaterials

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Chitosan is a natural polysaccharide, polycationic copolymer of glucosamine and N-acetyl glucosamine, already employed in multiple biomedical applications.[1] Unique physicochemical properties include biocompatibility, biodegradability, antimicrobial activity and ease of chemical derivatization, allowing extensive tuning of biological and mechanical properties. In this work, chitosan was employed for the development of antimicrobial and diagnostic nanomaterials.

Water-soluble chitosan has been selected as scaffold for *Pseudomonas Aeruginosa* LecB targeting multivalent antimicrobials. Novel epoxy-mannoside-based sulfonates and sulfoximines suitable for conjugation to chitosan were synthetized and tested. The resulting polymer allowed the formulation of self-assembled nanoparticles with enhanced affinity towards LecB, showing cluster effect and building supramolecular structures through self-assembly of proteins mediated by small molecules.

Higher M.W. chitosan was combined with γ-PGA and formulated as self-assembled polyelectrolyte complexes. Polymer were functionalized for subsequent chemoselective decoration with a ligand for specific targeting of pancreatic β-cells and different detecting agents (PET, MSOT).[2,3] Obtained nanoparticles are a promising candidate as versatile *in vivo* probes, allowing the labelling of multiple entities in a multimodal fashion. The properties of derivatized polymers and nanoparticles have been characterized, and their biocompatibility was examined in vitro and in vivo. Biodistribution has been tested by PET in mice and pigs with Ga-68 labelled nanoparticles.

Keywords: biomaterials, nanoparticles, multivalency, chitosan, in vivo imaging, antimicrobials

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Speaker

Abstract Book

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Prof Tarja Malm is Professor (tenure track) in Molecular Neurobiology and the head of the Neuroinflammation research group at the A.I.Virtanen Institute, University of Eastern Finland. She obtained her PhD in 2006 in Neurobiology with the focus glial cell biology and carried out her postdoctoral training at the Case Western Reserve University, USA. Her research focuses on understanding how and why microglia become malfunctional in different neurodegenerative diseases and how environmental factors, such as infections and air pollution alter microglial functionality and microglia-neuron interactions. Her group uses interdisciplinary approaches and develops novel, human based models to find therapeutic strategies to combat brain diseases. Her research group has pioneered development of methodologies to differentiate microglia and microglia containing cerebral organoids from human induced pluripotent stem cells. These models enable investigation of air pollution effects on microglia functionality and microglia-neuron interaction.

Traffic related air pollution alter human microglial functions

Microglia are the native immune cells of the central nervous system (CNS) and play key roles in synaptic pruning, lipid metabolism, and immune response. Recent experimental data suggest that traffic-related particulate matter (PM) exposure affects the CNS and microglial functions, thereby contributing to the prevalence of CNS diseases. Majority of the studies have been carried out using rodent models of microglia. However, human and mouse microglial responses have been reported to be considerably different, which is likely reflected also to their responses to PM.

We took advantage of induced pluripotent stem cell (iPSC) -derived microglia (iMGLs) allowing us to explore an array of human microglia-specific functional responses to traffic related PMs *in vitro*, including survival, cytokine secretion, phagocytosis and metabolis responses. Our study indicate that traffic-related air pollutants alter the function of human microglia, which could contribute to adverse effects in brain and cognition over time. This study demonstrates human iPSC-microglia as a valuable tool to study microglia-specific responses to environmental factors.

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Curriculum vitae

Julian Quodbach is a pharmacist by training and obtained his PhD at Heinrich Heine University Düsseldorf, Germany. Since 2015 he is researching 3d printing as manufacturing tool for pharmaceutical products. After a postdoc at the Drug Delivery Group at Uppsala University, Sweden in 2019, Julian Quodbach returned to Düsseldorf to continue his work on 3D printing. In January 2022, Julian joined the Utrecht Institute for Pharmaceutical Sciences at Utrecht University, The Netherlands as Assistant Professor. There, he connects his academic work on 3D printing of medicines with clinical applications.

Can 3D printing solve poorly soluble drugs?

Abstract: Pharmaceutical manufacturing is facing enormous challenges in the next years. Precision medicine, the principle of supplying patients with the right drug in the right dose at the right time, is gaining momentum due to cheaper and better available genotyping approaches and an ever improving understanding of pharmacological mechanisms. The translation of this approach will require the manufacturing of small or even individual batches of medicines, a task current pharmaceutical processes are not designed for. In parallel, the number of poorly soluble drugs is greatly increasing. An estimated 80 – 90 % of drugs in the development pipelines are poorly soluble and require the use of enabling technologies [1]. While several solubility enhancing strategies are established, it is unclear how these could be translated to treatments according to precision medicine.

3D printing is an emerging set of technologies for the manufacturing of medicines that that demonstrated its usefulness for manufacturing for small and individual batches. In this presentation, I will explore if poorly soluble drugs can be formulated for 3D printing technologies. If such a coupling of technologies is not feasible, modern drugs cannot be used for advanced treatment regimen.

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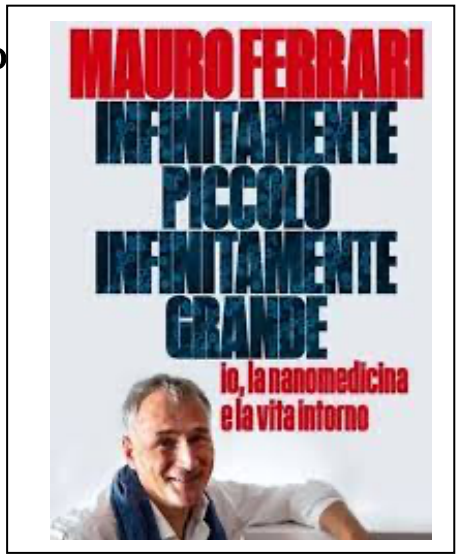

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Infinitamente Piccolo, Infinitamente Grande

Abstract: I will give you a subtitle: My 30 years of failures trying to cure metastatic disease to lungs and liver. I think we are about to get there, but man it has been a tough ride. The good is that the various failures have given rise to many good developments, which one way or the other have ended up being beneficial to many patients worldwide – but what I am after is a real breakthrough against lung and liver mets – because they are the number one cause of cancer death. Of course no guarantee that what we are doing will work, but yes guarantee that we will give it all we got – that is the mission. I now have 6 jobs, apparently different, but really all part of the same mission – namely my work at BrYet Pharma, Arrowhead Pharma, the University of Washington, plus the music, the all-truth narrative books, the movies. Maybe I will sing for you – I am not sure, it will depend on circumstances. Looking forward to seeing you there and especially answering questions.

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Energy at origins: The importance of the environment in early evolution

All theories for the origin of life require a source of energy to promote primordial chemical reactions[1-6]. Evidence for the nature of energy at origins should be preserved in the biochemical reactions of life itself, and changes in free energy, ∆G, should help specify the source. Calculating values of ∆G across the conserved and universal core of 402 individual reactions that synthesize amino acids, nucleotides and cofactors from H_2, CO_2, NH_3, H_2S and phosphate in modern cells reveals that 95–97% of these reactions are exergonic at pH 7-10 and 80-100°C with H_2 replacing biochemical reductants under nonequilibrium conditions[1]. While 23% of the core's reactions involve ATP hydrolysis, 77% are ATP-independent, thermodynamically driven by ∆*G* of reactions involving carbon bonds. These 402 reactions trace to the last universal common ancestor (LUCA), and reveal that synthesis of LUCA's chemical reactions did not require external energy inputs such as lightning or UV-light, but did require the H_2-rich conditions of serpentinizing hydrothermal systems[1-6]. The biosynthetic reactions of LUCA uncover a natural thermodynamic tendency of metabolism to unfold from energy released by reactions of H_2, CO_2, NH_3, H_2S, and phosphate[1] — a bioenergetic relic of the hydrothermal environment within which metabolism arose.

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Curriculum vitae: Dr. Tedesco is technologist at Biotechnological Department of Stazione Zoologica Anton Dohrn in Naples, a worldwide recognized center for marine studies. During his PhD at the University of Naples, Dr. Tedesco has focused his attention on psychrophilic bacteria, adapted to low temperatures as source of new enzymes and molecules with antimicrobial activities. He has established a biodiscovery pipeline for new bioactive compounds starting from the isolation of psychrophilic bacteria to the purification and characterization of the active molecules. After obtaining his PhD in 2016 he spent a semester in the laboratory of Prof. Marla Trindade at the University of Western Cape in South Africa, where he gained interest in genome mining of biosynthetic gene clusters for natural products and their heterologous expression and for synthetic biology and metabolic engineering. He had then the chance to increase his knowledge in this topic during his post-doc position at the Toulouse Biotechnology Institute in Toulouse, France. There, he worked mainly on the Oligomet Project whose final goal was to generate a versatile microbial chassis to produce oligosaccharides. In 2021, Dr Tedesco started his job as technologist at the SZN where his activities aim at the valorisation of biotechnological potential of marine microorganisms.

Biosurfactants from extreme environments: from discovery to biotechnological applications

Bio-surfactants are secondary metabolites produced by many microbial species, endowed with important biological activities that possess advantages compared to their synthetic counterparts. They found applications especially in food cosmetic and pharmaceutical industries as ingredients or active principles and are also applied for bioremediation. Therefore, there is an interest in discovering new biosurfactants and in this perspective, bioprospecting from unexplored and extreme environments has proven to be an excellent strategy to obtain new compounds. Marine bacteria have emerged as a promising source of new biosurfactants with different structures and properties, due to the peculiar and extreme environmental conditions[1,2]. In the last years our research group has set-up of a discovery pipeline for biosurfactant from marine bacteria. This pipeline starts with isolation of new bacteria from marine sediments to the identification of new molecules using different screening techniques and analytical instrumentation. Using this strategy, we have identified many biosurfactants-producer strains, purified and characterized several mixtures showing interesting anti-infective activities against pathogen bacteria and viruses [3,4]. Some of these mixtures are currently under further development for the generation of products of biomedical interest. Finally, we have implemented our pipeline with pathways engineering and heterologous expression to achieve higher production rates and obtain new products [5].

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2000: *M. Sc.* Degree in Chemistry and Pharmaceutical Technology (full marks) at University of Bari (Italy) and awarded as "Best graduate of Academic Year" of the Faculty.

2001-2004: PhD in "Medicinal Chemistry" - University of Bari. Thesis in Organic Chemistry: "*New synthetic methodologies for the synthesis of pharmacologically active isoxazoles*", with a six months stage at Centre of Studies on Aging (Chieti, Italy) for the study of new heterocyclic NSAIDs with Prof. P. Patrignani.

2004-2006: PostDoc fellow at the Department of Pharmacy - University of Bari, working at interdisciplinary projects in the field of five-membered ring heterocycles: *Preparation and spectroscopic characterization of potent and selective COX inhibitors.*

2006-2021: Assistant professor of Organic Chemistry at the University of Bari, teaching Organic Chemistry at the Faculty of Pharmacy, and achieving the National Scientific Habilitation in 2016.

From 2022 she is Associate Professor of Organic Chemistry of the Department of Pharmacy-Drug Sciences - University of Bari.

Her current research is focused on the development of new green approaches for the preparation of fine chemicals and active pharmaceutical compounds (APIs), moving from biocatalysis to organometallic chemistry employing water or “deep eutectic solvents” as promising green reaction media.

Water and *Deep Eutectic Solvents* as promising reaction media toward new greener approaches for the preparation of fine chemicals and APIs

Energy and environmental problems are increasingly affecting our daily life, and the discovery of more sustainable materials, technologies and processes represents a great challenge for academic and industrial scientific research.[1, 2]

However, synthetic chemistry is still highly dependent on petroleum-derived raw materials and a gradual shift to more sustainable processes, with new materials and chemicals derived from biological and renewable sources is highly desiderable.

In this contribution, the development of new, more ecological approaches for the synthesis of fine chemicals and active pharmaceutical ingredients (APIs) by replacing traditional VOCs (Volatile Organic Compounds) with unconventional reaction media will be discussed.[3]

Particular attention will be paid to the use of water and deep eutectic solvents (DES), an emerging class of neoteric solvents which have found successful applications in several scientific fields (e.g. extraction methods, metal-, bio- and organo- catalysis, photosynthesis, etc.),[4] thanks to the tunability of their physicochemical properties, minimal ecological footprint (negligible vapour pressures, non-flammability, easy recycling and biodegradability), ease of preparation and cost-effectiveness, in compliance with the principles of Green Chemistry,[5] towards safer, cheaper and more environmentally friendly preparative methodologies.

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Pablo I. Nikel-Mayer earned a Ph.D. in Biotechnology and Molecular Biology (2009) in Buenos Aires, Argentina. During graduate school, his research focused on repurposing several two-component signal transduction systems in *Escherichia coli* to produce biopolymers and biofuels. After receiving training in ^{13}C-based quantitative physiology technologies in USA (Rice University, supported by the ASM), Pablo moved to Europe in 2011 as a post-doctoral fellow in Prof. de Lorenzo's laboratory in Madrid, funded by the European Molecular Biology Organization (EMBO) and the Marie Skłodowska-Curie Actions (MSCA) of the European Commission. During his post-doctoral training, he came across the world of environmental bacteria—and, in particular, that of *Pseudomonas putida*. Inspired by the unique possibilities that this bacterium offers for bioengineering, he is now leading the *Systems Environmental Microbiology* Group at DTU Biosustain. Pablo's team aims at rewriting *P. putida*'s core biochemistry through synthetic metabolism for biosynthesis of novel compounds with a focus on new-to-nature fine chemicals (www.sem-cfb.com). The ultimate ambition of this research programme is expanding the very limits of microbial biochemistry—granting access to compounds that, as of today, are exclusively produced *via* traditional chemistry nowadays. Pablo is also the coordinator of the H2020 project *SinFonia* (www.sinfoniabiotec.eu).

Daring the limits of chemistry in living cells by engineering synthetic metabolism for biofluorination

Fluorine is a key element for the synthesis of molecules broadly used in medicine, agriculture and materials. Adding fluorine atoms onto organic structures is a unique strategy for tuning molecular properties—yet organofluorines are rarely found in Nature, and approaches to integrate fluorometabolites into the chemistry of living cells are scarce. Here, I will also discuss how synthetic metabolism[1] can be implemented to expand the chemical landscape of bacteria, thus providing alternative biosynthetic strategies for fluorinated building-blocks. This general approach will be illustrated by showing how synthetic gene circuits can be engineered in the platform bacterium *Pseudomonas putida* for organofluorine biosynthesis[2]. To this end, fluoride-responsive riboswitches and orthogonal RNA polymerases were used to drive biochemical reactions needed for *in vivo* biofluorination. Biosynthesis of fluoronucleotides and fluorosugars in engineered *P*. *putida* is demonstrated with mineral fluoride both as the only fluorine source (i.e. as a substrate of the pathway) and as inducer of the synthetic circuit.

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Dina Zielinski is a molecular and computational biologist based in Paris. Most of her research has been in human genetics and genomics, from decoding mutations in cancer and rare diseases to encoding digital data in DNA. She studied biology and French at NYU where she received her bachelor's degree. Dina started her career as a molecular biologist at the Whitehead Institute/MIT where she worked on bridging molecular and computational strategies in human genetics. Later, she moved to the New York Genome Center and Columbia University where she was inspired to focus on bioinformatics to bring biological data to life. While at the Institut Curie, she completed her MSc in bioinformatics at the Université de Paris, followed by a PhD in genomics and biomedical informatics at Sorbonne Université. She is currently a senior scientist in the Paris Transplant Group (INSERM) where she applies machine learning approaches in organ transplantation to improve patient care. Dina was selected as a 2020 National Academy of Sciences Kavli Frontiers of Science Fellow. Her work has been profiled by The Atlantic, BBC, Forbes, NPR, STAT, Wall Street Journal, and WIRED, and her TEDxVienna talk on DNA Storage, published on TED.com, has received nearly 2 million views.

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A future for digital data in nature’s oldest storage device

Big data has become a big problem. Our world is driven by information yet no storage device can keep pace with the growing data deluge. Archival storage relies on magnetic tape, which lasts tens of years at best and is costly to maintain. All the world's annual data would weigh less than a sugar cube if stored on nature's oldest storage medium: DNA. Not only is DNA incredibly dense and durable, it's possible to read, write, and copy. Information can be copied trillions of times on DNA and retrieved without error. Every new manmade storage device requires a new way to read it and eventually becomes obsolete or unreadable. This will never be the case with DNA. Recent developments in synthesis, encoding, and decoding schemes have advanced the field at breakneck speed. While traditional media cannot compete with DNA in density, longevity, and parallel computing, there are theoretical and practical barriers to DNA storage and computing. However, DNA does not necessarily have to compete with conventional storage media or silicon-based computers. We can instead harness the unique properties of DNA and other small molecules for specific applications.

Dan Peer, PhD
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Dan Peer is a Professor and the Director of the Laboratory of Precision NanoMedicine at Tel Aviv University (TAU). He is also the Vice President for Research and Development at Tel Aviv University. From 2017 - Present, he is the Founding and Managing Director of the SPARK program of Translational Medicine at TAU.

Prof. Peer's work was among the first to demonstrate systemic delivery of RNA molecules using targeted nanocarriers to the immune system and he pioneered the use of RNA interference (RNAi) in immune cells. His lab was the first to show systemic, cell specific delivery of modified mRNA to cells to induce therapeutic gene expression of desired proteins within the immune system that has enormous implications in cancer, inflammation and infection diseases (e.g. COVID 19 mRNA vaccines). In addition, his lab was the first to show high efficiency, systemic, cell specific therapeutic genome editing in cancer.

Prof. Peer has more than 130 pending and granted patents. Some of them have been licensed to several pharmaceutical companies and one is currently under registration (as a new biological drug in Inflammatory Bowel Disease). In addition, based on his work, five spin-off companies were generated aiming to bring innovative personalized medicine into clinical practice.

Prof. Peer received more than 30 awards and honors and he serves on the scientific advisory board and as Board Member of more than 15 companies, and on the editorial board of more than 20 journals. He is also the chairman of Ramot, TAU Technology Transfer Company, Chairman of TAU Venture, Chairman of NeoVac Ltd. in Oxford UK, and Chairman of Tura Innovations Ltd.

RNA Therapeutics is Going Beyond the Liver:

From Gene Silencing to Gene Editing

Accumulating work points out relevant genes and signaling pathways hampered in human disorders as potential candidates for therapeutics**.** Developing nucleic acid-based tools to manipulate gene expression, such as siRNAs, mRNA and genome editing strategies, open up opportunities for personalized medicine. Yet, although major progress was achieved in developing RNA targeted delivery carriers, mainly by utilizing monoclonal antibodies (mAbs) for targeting, their clinical translation has not occurred. In part because of massive development and production requirements and high batch-to-batch variability of current technologies, which relies on chemical conjugation. Here we present a self-assembled modular platform that enables to construct theoretically unlimited repertoire of RNA targeted carriers. The platform self-assembly is based on a membrane-anchored lipoprotein, incorporated into RNA-loaded novel, unique lipid nanoparticles that interact with the antibody Fc domain. We show that a simple switch of 8 different mAbs, redirects specific uptake of siRNAs by diverse leukocyte subsets *in vivo.* The platform therapeutic potential is demonstrated in an inflammatory bowel disease model, by targeting colon macrophages to reduce inflammatory symptoms, and in Mantle Cell Lymphoma xenograft model, by targeting cancer cells to induce cell death and improve survival. In addition, I will discuss novel approach for delivering modified mRNA to specific cell types *in vivo* utilizing this platform. I will also share some data on mRNA vaccines for COVID19 and Finally, I will share new data showing very high efficiency genome editing in glioma and metastatic ovarian cancer. This modular delivery platform can serve as a milestone in turning precision medicine feasible.

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