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6TH BIOCHIMICA DEI TUMORI WORKSHOP

UNTANGLING THE MOLECULAR AND METABOLIC NETWORKS IN CANCER PROGRESSION

Bologna
July 9-10th, 2026



Abstract book

UNTANGLING THE MOLECULAR AND METABOLIC NETWORKS IN CANCER PROGRESSION

6th Workshop of the SIB group "Tumor Biochemistry"
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Oral Presentation

GTP homeostasis supports mitochondrial *cristae* remodeling and pancreatic tumor progression

Francesca Bozzetto[1], Carlotta Paoli[2], Vittoria Michelotto[3], Roberta Noè[4], Marco Fantuz[5], Beatrice Calciolari[6], Nicla Lorito[7], Elisa Giovannetti[8], Vincenzo Corbo[9], Andrea Morandi[10], Ildiko Szabo[11], Rushika Perera[12], Luca Scorrano[13], Alessandro Carrer[14]

[1]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; francesca.bozzetto.1@studenti.unipd.it; francesca.bozzetto@vimm.it
[2]Department of Biology, University of Padova, Italy; carlotta.paoli@unipd.it
[3]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; vittoria.michelotto@studenti.unipd.it; vittoria.michelotto@vimm.it
[4]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; roberta.noe@studenti.unipd.it; roberta.noe@vimm.it
[5]Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; marco.fantuz@vimm.it
[6] Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; beatrice.calciolari@vimm.it
[7]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Firenze, Italy; nicla.lorito@unifi.it
[8]Fondazione Pisana per la Scienza ONLUS, San Giuliano Terme (PI), Italy; elisa.giovannetti@gmail.com
[9]Department of Engineering for Innovation Medicine, University of Verona, Italy; vincenzo.corbo@univr.it
[10]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Firenze, Italy; andrea.morandi@unifi.it
[11]Department of Biology, University of Padova, Italy; ildiko.szabo@unipd.it
[12]Department of Anatomy, University of California San Francisco, San Francisco, CA, USA; rushika.perera@ucsf.edu
[13]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; luca.scorrano@unipd.it; luca.scorrano@vimm.it
[14]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; alessandro.carrer@unipd.it; alessandro.carrer@vimm.it

Background: Carcinogenesis of pancreatic ductal adenocarcinoma (PDAC) occurs through multi-step progression, in that tumor onset is preceded by the formation of increasingly dysplastic lesions that can evolve into frank carcinoma. Understanding how PDAC develops is critical to improve early diagnosis. Progression from neoplastic lesions to carcinoma hinges on the accumulation of genetic mutations but also to step-wise metabolic reprogramming, which includes changes in mitochondrial structure and function.

Aim: To dissect the role of mitochondria in PDAC progression we employed a mitochondrial-proteomic screening to find deregulated proteins between the first and the latest stages of pancreatic cancer.

Results: We identified two highly-interconnected proteins upregulated in the aggressive stages of PDAC: the nucleotide diphosphate kinase NME4 and the guanine nucleotide exchange factor RCC1L. Silencing of either factor dampened the cellular respiratory capacity but also significantly impacted whole-cell metabolism. Mechanistically, NME4 and RCC1L cooperate to increase the activity of Optic Atrophy Protein 1 (OPA1), one of the master regulators of mitochondrial ultrastructure. In fact, PDAC cancer cells exhibit tighter cristae, which are widened by silencing of either NME4 or RCC1L. We also demonstrated that OPA1 elevation accelerates PDAC carcinogenesis in autochthonous mouse modes, while OPA1 targeting suppress tumor growth in vitro and in vivo. Moreover, reducing NME4 levels induces tumor-associated inflammation in a STING-independent manner, inducing tumor immunogenicity.

Conclusions: We demonstrated that NME4, RCC1L and OPA1 are functionally linked to promote PDAC and represent novel metabolic vulnerabilities. We also identified this cluster to impact mitochondrial structure and function by regulating mitochondrial GTP dynamics.

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Oral Presentation

MLK4 regulates mitochondrial dynamics and metabolic rewiring in pancreatic cancer

Noemi Ghiglione[1], Andrea Costamagna[1], Luca Gozzelino[2,3], Maria Chiara De Santis[1], Damiano Abbo[1], Lorenzo Prever[1], Emilio Hirsch[1], Paolo Porporato[1], Marta Giacomello[4], Marcello Manfredi[5], Andrea Morandi[6], Elisa Giovannetti[7], Vincenzo Corbo[8], Giuseppe Ferrauto[1], Miriam Martini[1]

[1]Molecular Biotechnology Center, Dep. of Molecular Biotechnology and Health Sciences, University of Turin, Italy; noemi.ghiglione@unito.it, a.costamagna@unito.it, mariachiara.desantis@unito.it, damiano.abbo@unito.it, lorenzo.prever@unito.it, emilio.hirsch@unito.it, paolo.porporato@unito.it, giuseppe.ferrauto@unito.it, miriam.martini@unito.it
[2]Italian Institute for Genomic Medicine, Candiolo, Italy; gozzelino.fellow@iigm.it
[3]Candiolo Cancer Institute, FPO – IRCCS, Candiolo, Italy; gozzelino.fellow@iigm.it
[4]Dep. of Biology, University of Padua, Padua, Italy; marta.giacomello@unipd.it
[5]Dep. of Translational Medicine, University of Piemonte Orientale, Novara, Italy; marcello.manfredi@uniupo.it
[6]Dep. of Experimental and Clinical Biomedical Sciences 'Mario Serio', University of Firenze, Italy; andrea.morandi@unifi.it
[7]Department of Medical Oncology, Lab of Medical Oncology, Cancer Center Amsterdam, Amsterdam UMC, VU University Medical Center, Amsterdam, The Netherlands; e.giovannetti@amsterdamumc.nl
[8]Dep. of Engineering for Innovation Medicine (DIMI), University of Verona, Verona, Italy; vincenzo.corbo@univr.it

Background: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with poor prognosis due to the limited early diagnostic tools and treatments. Its dense desmoplastic stroma limits nutrients and oxygen availability, promoting, together with KRAS signaling, a metabolic rewiring toward glycolysis. However, the mechanisms coordinating stress adaptation, mitochondrial dynamics and metabolic plasticity remain poorly understood. Mixed lineage kinase 4 (MLK4), a member of the MAP3K family implicated in JNK-dependent stress signaling pathways, may function at the interface between stress adaptation and actin dynamics. While downregulated in nearly 20% of patients, the role of MLK4 in PDAC progression remains unclear.

Aim: This project investigates MLK4's role in PDAC and the molecular mechanisms through which it regulates cellular processes.

Results: MLK4 localizes near mitochondria and its loss induces mitochondrial fragmentation, impaired oxidative phosphorylation and enhanced glycolytic activity. MLK4-deficient cells display increased glucose uptake, *in vitro* and *in vivo* lactate secretion, and higher sensitivity to glycolytic perturbation, indicating reduced metabolic flexibility. Interactome analysis identified non-muscle myosin MYH14, a regulator of actomyosin- dependent mitochondrial fission, as a novel MLK4 interactor, suggesting that MLK4 coordinates cytoskeletal dynamics and mitochondrial function. Moreover, MLK4 loss promotes invasiveness *in vitro* and in zebrafish and mouse models.

Conclusions: These findings suggest that MLK4 sustains mitochondrial integrity and metabolic homeostasis, restraining metabolic adaptation and PDAC progression. The metabolic rewire induced by MLK4 loss may represent a therapeutic vulnerability in pancreatic cancer.

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Oral Presentation

Metabolic Reprogramming in Lung Cancer: Tracing the Transition from Primary Tumor to Circulating Tumor Cells and Metastasis

Simone Serrao[1], Benedetta Gerosa[2], Marta Nobile[1], Eleonora Bossi[1], Ilaria Alberico[2], Giulia Bertolini[2], and Giuseppe Paglia[1]

[1]Department of Medicine and Surgery, University of Milano Bicocca, Via Raoul Follereau 3, 20854, Vedano al Lambro (MB), Italy; simone.serrao@unimib.it, m.nobile13@campus.unimib.it, e.bossi13@campus.unimib.it, giuseppe.paglia@unimib.it.
[2]Unit of Epigenomics and Biomarkers of Solid Tumors, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, 20133, Milano, Italy; benedetta.gerosa@istitutotumori.mi.it, ilaria.alberico@istitutotumori.mi.it, giulia.bertolini@istitutotumori.mi.it

Background: Circulating tumor cells (CTCs) represent the seeds of metastasis; however how tumor cell metabolism is rewired from primary tumor (PT) to CTCs and metastatic cells (Met) remains unclear.

Aim: We aim to define stage-specific metabolic flux signatures across the metastatic cascade in non-small cell lung cancer (NSCLC) by using flow cytometry and isotope tracing mass spectrometry in PT, CTCs and Met cells isolated from patient-derived xenograft model and cultured in vitro using fully-labelled ^{13}C-glucose, ^{13}C-glutamine and ^{13}C-palmitate.

Results: Isotope tracing data show a distinct metabolic flux for each population and highlight that the transition from PT to CTC and Met involves different use of glycolysis, purine metabolism, glutamine-dependent anaplerosis, and fatty-acid β-oxidation. PT cells show preferential glucose-derived glycolytic flux, together with pyruvate and hypoxanthine utilization, consistent with a glycolytic and purine-scavenging phenotype. CTCs display increased CD36 and enhanced ^{13}C-palmitate contribution to acetyl-CoA/TCA intermediates, indicating activation of fatty-acid uptake and oxidation. CTCs also show increased labelling of adenine nucleotides, suggesting increased pentose-phosphate/purine recycling to sustain ATP turnover. CD73 increases in PT and CTCs but not in Met cells, supporting a transient purinergic/adenosine-associated phenotype during local growth and circulation. In contrast, Met cells show increased ^{13}C-glutamine/α-ketoglutarate contribution to TCA cycle, indicating glutamine-dependent anaplerosis after colonization.

Conclusions: The data reveal a stage-resolved metabolic reprogramming in lung cancer progression: glycolytic/purine-scavenging PT cells, CD36-high lipid-fueled and purine-recycling CTCs, and α-ketoglutarate-dependent Met cells. This flux-based model identifies CTCs lipid and purine metabolism as potential checkpoints to intercept metastatic dissemination.

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Oral Presentation

The RNA-binding protein SDOS/TIRR shapes riboregulation, DNA damage response and interferon signaling in breast cancer cells

Anna Di Micco[1], Rosario Avolio[1], Franca Esposito[1] and Danilo Swann Matassa[1]

[1]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II
daniloswann.matassa@unina.it

Background: Breast cancer (BC) is the most frequently diagnosed cancer and the second leading cause of death in women. In BC cells, Syndesmos (SDOS/NUDT16L1/TIRR) is a key regulator of 53BP1-mediated DNA damage response: its overexpression prevents 53BP1 function, leading to PARP inhibitor resistance in BRCA-mutated tumors. SDOS is also an RNA-binding protein that associates with polysomes and regulates translation, and SDOS-interacting RNAs reduce its 53BP1 inhibition,indicating that these functions are coupled.

Aim: By using OOPS, RIP, polysome profiling and PLA we aimed at characterizing SDOS interactions with protein partners and RNA targets and defining the contribution of its cytoplasmic roles to the DNA damageresponse.

Results: We found that SDOS interacts with the major autophagy receptor SQSTM1/p62 and influences the riboregulation exerted by the small noncoding vault RNAs on its activity: upon SDOS silencing, the binding between p62 and vtRNA 1-1 is reduced, and autophagy is increased, in response to DNA damage. SDOS silencing also dramatically increases the expression of interferon-stimulated genes, particularly ISG15, thus mimicking DNA damage-induced interferon response. Of note, we found that SDOS binds the IRF9 transcript, a major ISG15 regulator, candidating SDOS as a key modulator of the interferon pathway. SDOS thus contributes to the DDR at multiple levels, through the regulation of transcripts encoding stress-responsive proteins and the riboregulation of their activity. These molecular mechanisms account at least in part for its clinical relevance: bioinformatic analyses show that high SDOS expression correlates with better patient survival and enhanced response to therapy in triple-negative and Her2+ BC.

Conclusions: We propose that SDOS overexpression promotes tumorigenesis by inducing genomic instability, while representing a vulnerability in advanced tumors treated with chemotherapy because of its inhibitory effects on pro-survival mechanisms.

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Oral Presentation

The chaperone TRAP1 couples glutaminolysis and aspartate availability to sustain melanoma cells invasion

Claudio Laquatra[1], Alessia Magro[1], Agata Pia Parabola[1], Raquel Branas Casas[2], Marco Fantuz[3], Denis Komarov[4], Natascia Tiso[2], Giorgio Colombo[4], Daniela De Zio[5] and Andrea Rasola[1]

[1]Department of Biomedical Sciences, University of Padova, Padova, Italy; claudio.laquatra@unipd.it, alessia.magro.1@studenti.unipd.it, agatapia.parabola@studenti.unipd.it, andrea.rasola@unipd.it
[2]Department of Biology, University of Padova, Padova, Italy; natascia.tiso@unipt.it, raquel.branascasas@unipd.it
[3]Veneto Institute of Molecular Medicine (VIMM), Padova, Italy, marco.fantuz@vimm.it
[4]Department of Chemistry, University of Pavia; g.colombo@unipv.it, denis.komarov01@univerditadipavia.it
[5]Institute of Molecular Medicine (IMM), University of Southern Denmark (SDU), Odense, Denmark

Background: Melanoma is a highly aggressive malignancy characterized by extensive metabolic rewiring driven by oncogenic signaling; yet, the mechanisms linking mitochondrial function to tumor progression remain poorly defined. Enhanced glutamine (Gln) utilization is a central metabolic adaptation that sustains biosynthetic programs in cancer cells, but its regulation and therapeutic exploitability in melanoma are not fully understood. Recent studies have identified the mitochondrial chaperone TRAP1 as a potential regulator of Gln metabolism, yet a comprehensive analysis of this adaptation and its relevance to melanoma progression are entirely lacking.

Aim: This study delineates the mechanism by which TRAP1 regulates Gln usage and how this process supports melanoma growth and metastatic dissemination.

Results: By integrating biochemical, metabolic flux and transcriptomic analyses, we show that TRAP1 interacts with GLS1 to sustain glutaminolysis and TCA cycle anaplerosis. TRAP1 loss impairs GLS1 flux and induces a compensatory shift toward glucose-derived glutamate production, while causing a marked depletion of aspartate associated with uncoupling of the malate-aspartate shuttle. Under nutrient restriction, double TRAP1 and GLS1 silencing elicits synthetic lethality, leading to TCA cycle collapse and biosynthetic failure. This metabolic stress, together with aspartate depletion, activates the Integrated Stress Response and stabilizes ATF3, driving transcriptional repression of gene programs governing extracellular matrix remodeling and epithelial-to-mesenchymal transition, strongly impairing invasive and metastatic capacity in vitro and in vivo.

Conclusion: This work establishes TRAP1 as a key regulator of glutamine-glucose metabolic balance revealing a previously unrecognized link between mitochondrial chaperone function, metabolic plasticity and metastatic competence. Thus, the TRAP1-GLS1 axis emerges as a new metabolic vulnerability with potential therapeutic relevance in melanoma.

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Defective mitochondrial metabolism in mesenchymal stromal cells shapes a stress- adapted myelodisplastic-like bone marrow niche

C. Giallongo[1], D. Calvo[1], S. Giallongo[2], G. Conti[1], E. La Spina[1], A. Duminuco[3], Longhitano L.[1], G. Lazzarino[5], A. M. Amorini[1], A. M'hammed[6], F. Polito[6], S. Forte[7], G. Testa[8], D. Romano[3], A. Romano[8], F. Di Raimondo[8], G. Li Volti[1], D. Tibullo[1], G.A. Palumbo[4]

[1]Department of Biomedical and Biotechnological Sciences, Division of Medical Biochemistry, University of Catania, Catania
[2]Department of Medicine and Surgery, University of Enna "Kore", Enna
[3]UOC Ematologia con Trapianto di Midollo, A.O.U. Policlinico "G. Rodolico"-S. Marco, 95123 Catania
[4]Department of Medical Surgical Sciences and Advanced Technologies "GF Ingrassia", University of Catania, Catania
[5]Departmental Faculty of Medicine, UniCamillus-Saint Camillus International University of Health and Medical Sciences, Rome
[6]Department of Clinical and Experimental Medicine, University of Messina
[7]Mediterranean Institute of Oncology, Catania
[8]Department of General Surgery and Medical-Surgical Specialties, University of Catania

Background: Myelodysplastic syndromes (MDS) are clonal disorders of hematopoietic stem and progenitor cells (HSPCs) characterized by ineffective haematopoiesis and risk of transformation to acute myeloid leukaemia. Although the molecular mechanisms underlying HSPC dysfunctions are thought to be primarily cell-intrinsic, increasing evidence supports a role of extrinsic signals from the bone marrow niche, including mesenchymal stromal cells (MSCs). Cellular metabolism is essential for MSC function and may affect their interactions with HSPCs; however, the metabolic profile of MDS- MSCs has not been investigated yet.

Aim To determine whether metabolic dysfunctions of MDS-MSCs contribute to their impaired hematopoietic support.

Results Compared with healthy MSCs (h-MSCs), MDS-MSCs showed reduced oxygen consumption, higher AMP/ATP ratio, and mitochondrial depolarization. Although glycolytic activity was also reduced, it remained the prominent metabolic pathway.
To investigate MDS-MSC metabolic status impact in MSC-HSPC interaction, h-MSCs were pre-treated with ATP-synthase inhibitor oligomycin (OM) or glycolysis inhibitor 2 deoxy-D-glucose (2DG) and then cocultured with healthy HSPCs. $MSC_{OM,}$ but not $MSC_{2DG,}$ impaired HSPC function triggering oxidative stress and decreasing proliferation and clonogenic capacity. In MSCOM, RNA-seq confirmed the loss of key pathways necessary for a functional hematopoietic niche, along with the activation of the mitochondrial unfolded protein response. Interestingly, healthy mitochondria transplantation rescued MSC-HSPC interaction, improving MSCOM mitochondrial metabolism and their hematopoietic support capacity.

Conclusions: Mitochondrial impairment converts MSCs from a metabolically supportive stromal niche into stressed and self-preserving cells establishing a MDS-like niche. Notably, mitochondrial transplantation restored MSC function, highlighting mitochondrial delivery as a potential therapeutic strategy in MDS.

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IκB/NF-κB supports tumor progression through fatty acid oxidation and CD8+ T-cell impairment

Giovanna Carrà[1], **Sijia Gou**[1,5], Jessica Petiti[2], Alessandro Morotti[3], Alessio Menga[4] and Paolo Ettore Porporato[5]

[1]Department of Translational Medicine (DIMET), University of Eastern Piedmont, Via Solaroli 17, Novara, 28100, Italy.
[2]Division of Advanced Materials Metrology and Life Sciences, Istituto Nazionale di Ricerca Metrologica (INRiM), 10135 Turin, Italy
[3]Department of Clinical and Biological Sciences, University of Turin, Italy
[4]University of Eastern Piedmont, Department of Health Sciences - Center for translational Research on Autoimmune & Allergic Diseases - CAAD Corso Trieste 15/A, 28100 Novara – Italy
[5]Department of Molecular Biotechnology and Health Sciences-Molecular Biotechnology Center "Guido Tarone", University of Turin, Via Nizza 52, 10126 Turin, Italy;

Background: Metabolic rewiring is a hallmark of lung cancer and contributes not only to tumor growth but also to immune microenvironment modulation. Here, we investigated the role of the IκBα/p65 axis in regulating lipid metabolism and its impact on CD8+ T-cell function in lung cancer.

Methods: Bioinformatic analyses, in vitro functional assays, in vivo murine models, and genome-wide CRISPR/Cas9 screening were employed to dissect the metabolic and immunological effects of NF-κB/p65 activation in lung adenocarcinoma (LUAD).

Results: Constitutive NF-κB/p65 activation induced metabolic rewiring characterized by lipid accumulation, increased CPT1A expression, and enhanced oxidative metabolism. Mechanistically, p65 directly bound the CPT1A promoter, sustaining tumor metabolic adaptation. Pharmacological targeting of lipid metabolism significantly impaired the survival of p65-activated tumor cells, revealing a targetable metabolic vulnerability. Conditioned media from IκBα-silenced tumor cells induced increased lipid uptake, mitochondrial respiration, and lipid droplet accumulation in CD8+ T lymphocytes, associated with oxidative stress, metabolic switching toward FAO, and acquisition of exhaustion markers. Immune analyses of LUAD patient datasets further demonstrated that high p65 expression correlates with reduced CD8+ T-cell infiltration, enrichment of immunosuppressive populations, and accumulation of exhausted lymphocytes. Genome-wide CRISPR/Cas9 screening identified PCCB as a key regulator of T-cell metabolic fitness under tumor-induced lipid stress. PCCB silencing recapitulated the dysfunctional phenotype observed in T cells exposed to tumor-conditioned media, promoting oxidative metabolism and exhaustion. Moreover, SLC46A2 and FUT4 emerged as potential immunometabolic therapeutic targets. Notably, Sulfasalazine treatment significantly reduced T-cell exhaustion markers ex vivo, suggesting partial restoration of immune functionality.

Conclusions: Our study identifies a novel IκBα/p65-driven immunometabolic program linking tumor metabolic rewiring to immune dysfunction in lung cancer. These findings highlight how metabolic alterations in tumor cells shape CD8+ T-cell exhaustion and identify PCCB, SLC46A2, and FUT4 as potential therapeutic vulnerabilities. Importantly, pharmacological targeting with Sulfasalazine partially restored T-cell functionality ex vivo, supporting the potential of immunometabolic therapies as innovative treatment strategies for lung cancer.

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Oral Presentation

Aggressive Breast Cancer Cell-Derived Conditioned Media Induce Inflammatory and Metabolic Reprogramming of Stromal Fibroblasts toward a CAF-like State

Alessia Ciringione[1], Marina Marozzi[1], Mariana Gallo[2], Elena Ferrari[2], Martina Bonacini[3], Stefania Croci[3], Thelma Pertinhez[2], Federica Rizzi[1]

[1]Laboratory of Biochemistry, Molecular Biology and Oncometabolism, Department of Medicine and Surgery, University of Parma, Parma, Italy; alessia.ciringione@unipr.it; federica.rizzi@unipr.it
[2]Laboratory of Biochemistry and Metabolomics, Department of Medicine and Surgery, University of Parma, Parma, Italy.
[3]Unit of Clinical Immunology, Allergy and Advanced Biotechnologies, Azienda Unità Sanitaria Locale- IRCCS di Reggio Emilia, Reggio Emilia, Italy.

Background: The reprogramming of stromal fibroblasts into cancer- associated fibroblasts (CAFs) plays a critical role in breast cancer (BC) progression by promoting extracellular matrix (ECM) remodeling, angiogenesis, tumor cell migration and immune escape through paracrine signaling. However, it remains unclear whether and how the metastatic properties of BC cells affect the transition of fibroblasts from a normal to an activated state.

Aim: In this study, we investigated whether conditioned media (CM) derived from two BC cell lines with distinct metastatic potential, MDA-MB- 231 and MCF-7, drives the conversion of human fibroblasts toward a pro- tumorigenic phenotype, using molecular and metabolomic analyses to dissect the mechanisms of tumor-stroma crosstalk.

Results: The exposure to MDA-MB-231 CM led to increased secretion of inflammatory cytokines and chemokines, including IL-6, IL-8, ICAM-1, and MCP-1, and production of ECM proteins. Consistently, we observed the upregulation of FAP, a well-known marker for BC CAFs. These effects were moderate or absent when fibroblasts were treated with MCF-7 CM. In parallel, we conducted ^{1}H-NMR metabolomic profiling of culture media. Fibroblasts grown in MDA-MB-231 CM showed a more pronounced shift from oxidative phosphorylation to aerobic glycolysis than those cultured in MCF-7 CM. We also observed increased secretion of alanine and TCA intermediates that support cancer cell feeding and favor immune escape mechanisms. All these metabolic changes are concomitant with c-MYC upregulation.

Conclusions: Altogether, our findings suggest that soluble factors secreted by highly aggressive BC cells promote a functional reprogramming of normal fibroblasts toward a pro-tumorigenic, inflammatory phenotype, ultimately contributing to immune evasion and therapy resistance. Elucidating the symbiotic crosstalk between BC cells and their tumor microenvironment holds promise to open new avenues for targeted therapeutic interventions.

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Oral Presentation

Glutamate overload in Myelofibrosis microenvironment reshapes mesenchymal stromal cells metabolic and epigenetic profiles

Sebastiano Giallongo[1], Lucia Longhitano[2], Cesarina Giallongo[2], Jessica Ferrigno[2], Enrico La Spina[2], Andrea Duminuco[3], Vittorio Del Fabro[1], Francesco Bellia[2], Angela Maria Amorini[2], Claudia Di Giacomo[4], Nunzio Vicario[2], Ignazio Alberto Barbagallo[2], Giovanni Li Volti[2], Giuseppe Alberto Palumbo[5] and Daniele Tibullo[2]

[1]Faculty of Medicine and Surgery, University of Enna "Kore", 94100 Enna, Italy. Sebastiano.giallongo@unikore.it; Vittorio.delfabro@unikore.it.

[2]Department of Biomedical and Biotechnological Sciences, University of Catania, 95123, Catania, Italy. lucia.longhitano@unict.it; cesarina.giallongo@unict.it; jessica.ferrigno.1996@gmail.com; Francesco.bellia@unict.it; amorini@unict.it; nunziovicario@unict.it; ibarbag@unict.it; livolti@unict.it; d.tibullo@unict.it.

[3]Hematology Unit with BMT, A.O.U. Policlinico "G. Rodolico-San Marco", Via S. Sofia 78, 95123 Catania, Italy. Andrea.duminuco@gmail.com.

[4]Department of Drug and Health Sciences, University of Catania, Viale A. Doria 6, 95125 Catania, Italy. Claudia.digiacomo@unict.it.

[5]Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", University of Catania, 95123, Catania, Italy. Palumbo.gam@gmail.com.

Background: Myelofibrosis (MF), a clonal myeloproliferative neoplasm, is defined by bone marrow fibrosis, osteosclerosis and a severe inflammation of tumor microenvironment (TME). Here, mesenchymal stromal cells (MSCs) rewire their metabolic and epigenetic profiles to sustain malignant hematopoietic stem cells progression. However, the mechanism underlying this process is still unmet.

Aim: here, we dissected the role of glutamate, already reported to promote several cancers, in MF-TME.

Results: high-performance liquid chromatography analysis glutamate as the prominent enriched aminoacids in MF sera. In MSCs, glutamate overload triggers intracellular fumarate accumulation by increasing the expression of α-ketoglutarate dehydrogenase components and downregulating fumarate hydratase activity. Consequently, intracellular fumarate accumulation reshapes MSCs metabolic profile towards oxidative phosphorylation, also triggering mitochondrial ROS production. In vitro, glutamate and fumarate reshape MSCs epigenetic profile, in turn enriching 5-methylcytosine abundancies, along with fumarate associated epigenetic marker H3K36me2. Of note, these results were further confirmed in primary MF-MSCs. Besides their epigenetic roles, glutamate and fumarate induce MSCs senescence onset, as supported by assessing β-galactosidase activity and inflammatory gene expression. Furthermore, both metabolites promote collagen deposition, responsible for MF-associated fibrosis onset. Interestingly, the selective inhibition of metabotropic glutamate receptor 5 restrained glutamate and fumarate related outcomes both in vitro and in vivo.

Conclusions: these results highlight the prominent role of glutamate in MF pathogenesis, eventually proposing mGluR5 inhibition as a novel therapeutical approach to mitigate MF progression.

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Oral Presentation

The interaction of IF1 with the OSCP subunit of ATP synthase has a key anti-apoptotic role in lung and cervix cancers

Valentina Giorgio[1], Martina Grandi[1], Ida Schaldemose Nielsen[1], Valentina Del Dotto[1], Silvia Grillini[1], Riccardo Righetti[1], Giancarlo Solaini[1], Alessandra Baracca[1]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy; valentina.giorgio4@unibo.it

Background: The mitochondrial protein IF1 is the natural inhibitor of ATP synthase and binds the catalytic domain during the enzyme hydrolytic activity. In many tumors, IF1 is upregulated and acts as a pro-oncogenic factor through different mechanisms. Its overexpression was recently shown to promote the IF1 interaction in cervix cancer cells with the ATP synthase OSCP (oligomycin sensitivity conferring protein) subunit during oxidative phosphorylation.

Aim: Two mutant forms of the human mature IF1 have been recently identified, K57N and K57Q, that are recurrent in aggressive and therapy- resistant lung cancers (i.e. non-small cell lung cancer, NSCLC), and we aimed at characterizing their mechanisms of action.

Results: The transient and stable re-insertion of the *ATP5IF1* gene in IF1 KO HeLa cells allowed us to overexpress the wild-type or mutant IF1 proteins and was instrumental for dissecting their effects. IF1 mutants displayed an increased propensity to bind the OSCP subunit compared to wild-type IF1-expressing HeLa cells. These IF1 mutants did not affect proliferation, but altered the sensitivity of the PTP opening, resulting in an increased colony formation in soft agar.

Conclusions: Overall, our results suggest that the IF1 mutants found in lung patient biopsies might favor tumor growth by protecting cancer cells from PTP-dependent apoptosis, thus confirming the anti-apoptotic role of the IF1-OSCP interaction.

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UNTANGLING THE MOLECULAR AND METABOLIC NETWORKS IN CANCER PROGRESSION

6th Workshop of the SIB group "Tumor Biochemistry"
Bologna, July 9-10th, 2026

Oral Presentation

Glutamine synthetase as a metabolic rheostat of ovarian cancer progression and therapy response

Stefano Miglietta[1], Mariagrazia D'Agostino[1], Luna Laera[1], Carlos Sanchez-Martin[2], Francesca Moramarco[1], Martina Lanza[3], Giuseppe Colonna[4], Gennaro Cormio[5] and Alessandra Castegna[1]

[1]Department of Biosciences, Biotechnologies and Environment, University of Bari Aldo Moro, Bari, Italy; stefano.miglietta@uniba.it; mariagrazia.dagostino@uniba.it; luna.laera@uniba.it; f.moramarco13@studenti.uniba.it; alessandra.castegna@uniba.it
[2]Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy; carlos.sanchez.martin@uniroma2.it
[3]Department of Experimental Medicine, University of Salento, Lecce, Italy; martina.lanza@unisalento.it
[4]Gynecologic Oncology Unit, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy; g.colonna@oncologico.bari.it
[5]Department of Interdisciplinary Medicine, University of Bari Aldo Moro, Bari, Italy; Gynecologic Oncology Unit, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy; gennaro.cormio@uniba.it

Background: Single-cell datasets of high-grade serous ovarian cancer (HGSOC) indicate that glutamine synthetase (GS; GLUL) is poorly expressed by HGSOC cells and enriched in TAMs, where GS marks a protumoral state. Together with the glutaminolytic nature of HGSOC, this suggests that GLUL loss may shape cancer cell metabolism and crosstalk within the tumor microenvironment (TME).

Aim: We investigated whether GS controls metabolism, macrophage crosstalk and aggressiveness in HGS2 cells, a highly representative murine HGSOC model that recapitulates key features of the human disease and is suitable for studying HGSOC immunometabolism.

Results: *GLUL*-deficient HGS2 cells displayed enhanced glutamine catabolism, citrate accumulation and cholesterol-oriented lipid programs, associated with reduced mitochondrial fitness and altered carbon/nitrogen metabolism. Functionally, *GLUL*-deficient cells depended on extracellular glutamine and were vulnerable to its deprivation, indicating pronounced glutamine addiction. Moreover, they showed increased aggressiveness and enhanced platinum sensitivity *in vitro*, revealing potential therapeutic liabilities. To explore whether macrophages could buffer this constrained phenotype, we cocultured cancer cells with proinflammatory macrophages and observed that *GLUL*-deficient HGS2 cells reshaped macrophage polarization by reducing pro-inflammatory markers, suggesting macrophage- mediated rescue from glutamine vulnerability within the TME. Accordingly, *in vivo* intraperitoneal injection of *GLUL*-deficient HGS2 cells accelerated metastatic HGSOC growth in ascites and reduced survival compared with controls.

Conclusions: GS restrains a glutamine-addicted and aggressive immunometabolic state in HGSOC. *GLUL* loss links glutamine dependency, anabolic rewiring, macrophage reprogramming and metastatic progression, defining an aggressive but metabolically vulnerable tumor state with relevance for metabolism-informed therapeutic strategies.

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UNTANGLING THE MOLECULAR AND METABOLIC NETWORKS IN CANCER PROGRESSION

6th Workshop of the SIB group "Tumor Biochemistry"
Bologna, July 9-10th, 2026

Oral Presentation

Aldo-keto reductase 1C3 as a therapeutic target of 7-hydroxycoumarine-basedinhibitors to treat chemoresistance in aggressive and rare tumors

Gaia Vanzetti[1], Chiara Vigato[1], Cristina Tucciarello[2,3], Giorgia Giordano[2,3], Agnese Chiara Pippione[1], Roberta Bertolozzi[4,5], Luca Persano[6], Marco Lucio Lolli[1], Ymera Pignochino[2,3], Donatella Boschi[1], Simonetta Oliaro-Bosso[1].

[1]Dept. of Science and Drug Technology, University of Turin, via Pietro Giuria 9, 10125 Turin, IT
[2]Dept. of Clinical and Biological Sciences, University of Turin, Regione Gonzole 10, 10143 Orbassano, IT
[3]Candiolo Cancer Institute, FPO-IRCCS 10060 Candiolo, Turin, IT
[4]Dept. of Pharmaceutical and Pharmacological Sciences, University of Padova, via Marzolo 5-35121 Padova, IT
[5]Pediatric Research Institute, corso Stati Uniti, 4F-35127 Padova, IT
[6]Dept. of Women's and Children's Health, University of Padova, via Giustiniani 3-35128 Padova, IT

Background: Aldo-Keto Reductase 1C3 (AKR1C3) is an overexpressed enzyme in several other cancers, where it promotes tumor progression through the regulation of steroid hormone biosynthesis and prostaglandin signaling. AKR1C3 overexpression is also associated with chemoresistance, contributing to increased tumor aggressiveness and invasiveness, thus representing an attractive target for anticancer drug development.

Aim: To expand the portfolio of AKR1C3 inhibitors, we aimed to identify and optimize novel inhibitors based on a 7-hydroxycoumarin scaffold through AI-driven virtual screening, structure-based design, and structure–activity relationship (SAR) studies.

Results: AI-assisted virtual screening identified compound 1 (cpd 1), a selective and potent AKR1C3 inhibitor featuring a 7-hydroxycoumarin core. It showed strong enzymatic inhibition together with significant antiproliferative activity in the 22RV1 prostate cancer model, characterized by AKR1C3 upregulation. Moreover, cpd 1 demonstrated synergistic effects with doxorubicin in osteosarcoma (OS) cell lines, reducing the IC50 of doxorubicin in SAOS-2 cells from 80 to 2 nM upon co- treatment. In in-house generated doxorubicin-resistant HOS, KHOS, and MG63 cell lines, all exhibiting AKR1C3 overexpression, cpd 1 restored doxorubicin sensitivity and reactive oxygen species (ROS) production, ultimately inducing apoptotic cell death. Guided by the crystallographic studies, a SAR campaign was undertaken to design and synthesize novel analogues with improved inhibitory potency. The newly developed compounds were evaluated through in vitro enzymatic and cellular assays.

Conclusions: This study highlights the potential of 7-hydroxycoumarin-based AKR1C3 inhibitors as promising anticancer agents capable of overcoming chemoresistance and enhancing doxorubicin efficacy. The integration of AI- driven screening, structure-based optimization, and biological validation supports the further development of AKR1C3-targeted therapeutic strategies.

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6th Workshop of the SIB group "Tumor Biochemistry"
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Oral Presentation

Branched Oncolytic Peptides induce Immunogenic Cell Death and Antitumor Activity in Pancreatic Cancer

Alessandro Rencinai [1], Chiara Falciani [2]

[1]Department of Medical Biotechnologies, University of Siena, Italy; alessandro.rencin@student.unisi.it
[2]Department of Medical Biotechnologies, University of Siena, Italy; chiara.falciani@unisi.it

Background: Pancreatic ductal adenocarcinoma (PDAC) is refractory to immunotherapy due to an immune-excluded, suppressive microenvironment with dense stroma, poor CD8+ T-cell infiltration, impaired antigen-presenting activity, and suppressive myeloid populations. Combining tumor-selective cytotoxicity with immunogenic danger-signal release may help convert PDAC from "cold" to more responsive.

Aim: This study aimed to assess whether tetrabranched oncolytic peptides BOP7 and BOP9 selectively kill PDAC cells while promoting ICD- associated danger signaling and antitumor effects in preclinical models.

Results: BOP7 and BOP9 showed selective cytotoxicity against human PDAC cells, reduced PANC-1 migration and invasion, and induced ATP, HMGB1 and IFN-β release. In vivo, BOP9 reduced PANC-1 tumor grafting/growth. Unpublished data extend these findings to murine PDAC, showing potent activity against Pan02 cells, with reduced toxicity and binding toward non-tumor fibroblasts. Inhibition of apoptosis, ferroptosis, or necroptosis did not rescue Pan02 cells from BOP-induced death, supporting a membrane-destabilizing mechanism that is not primarily dependent on canonical regulated cell death pathways. BOP treatment also triggered ATP and HMGB1 release in Pan02 cells. In orthotopic PDAC-bearing mice, labelled BOP7 and BOP9 preferentially accumulated in primary tumors and metastases, with no relevant signal in healthy tissues. In an ICD-vaccination assay, Pan02 cells killed ex vivo with BOP7 or BOP9 protected a fraction of mice from tumor challenge. Preliminary therapeutic experiments indicated a reduction in tumor burden after BOP treatment.

Conclusions: These data support BOP7 and BOP9 as tumor-selective, membrane-active peptides combining direct PDAC cell killing with ICD- associated danger signaling and warrant further development as immunogenic anticancer agents.

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6th Workshop of the SIB group "Tumor Biochemistry"
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Oral Presentation

Metabolic plasticity underlies ferroptosis resistance driven by mutant p53 in pancreatic ductal adenocarcinoma

Adriana Celesia[1], Filippo Piccoli[1], Tian Wang[2], Yushu Hu[2], Federica Danzi[1,6], Antonino Aparo[3], Barbara Cisterna[4], Raffaella Pacchiana[1], Maria Teresa Scupoli[5], Claudio Luchini[6], Stefano Ugel[2], Massimo Donadelli[1]*, Alessandra Fiore[1]*

[1]Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biochemistry, University of Verona, Verona, Italy
[2]Department of Medicine, Section of Immunology, University of Verona Hospital Trust, Verona, Italy
[3]Research Center LURM (Interdepartmental Laboratory of Medical Research), University of Verona, Verona, Italy
[4]Department of Neurosciences, Biomedicine and Movement Sciences, Section of Anatomy and Histology, University of Verona, Italy
[5]Department of Engineering for Innovation Medicine, Section of Biomedicine, University of Verona, Verona, Italy
[6]Department of Diagnostics and Public Health, Section of Pathology, University and Hospital Trust of Verona, Verona, Italy

*These authors contributed equally

Background: Pancreatic ductal adenocarcinoma (PDAC) is a lethal and aggressive disease characterized by mutations in the tumor suppressor gene TP53, whose gain-of-function activities promote tumor survival. Ferroptosis, an iron-dependent form of regulated cell death driven by lipid peroxidation represents a potential therapeutic vulnerability. However, the influence of mutant p53 on ferroptosis susceptibility remains poorly understood.

Aim: We investigated how mutant p53 modulates ferroptosis in PDAC. We used CRISPR-Cas9 knockout models, overexpression systems, and multi-level metabolic and transcriptional analyses to uncover a mutant p53-dependent barrier to ferroptosis-based therapies and propose a novel combinatorial approach based on wild-type p53 pharmacological reactivation combined with ferroptosis to sensitize PDAC cells to ferroptosis. This therapeutic approach was validated both in vitro and in orthotopic murine model.

Results: Mutant TP53 deletion sensitized pancreatic cancer cells to ferroptosis, increasing oxidative stress, lipid peroxidation, and mitochondrial dysfunction. On the other hand, mutant TP53 expression preserved mitochondrial integrity and bioenergetic flexibility under ferroptotic stress. Transcriptomic analyses revealed a multi-layered adaptive program in mutant TP53 cells, including upregulation of antioxidant and metabolic genes, and activation of PI3K–AKT signaling, which is associated to a glycolytic shift to maintain ATP levels. Importantly, pharmacological reactivation of wild-type p53 disrupted this adaptive network, significantly increasing ferroptotic cell death both in vitro and in vivo.

Conclusions: Mutant p53 orchestrates transcriptional and metabolic plasticity that confers ferroptosis resistance in PDAC. Reactivating wild-type p53 in combination with ferroptosis induction overcomes this resistance, highlighting a therapeutically actionable vulnerability and supporting the development of combinatorial strategies.

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6th Workshop of the SIB group "Tumor Biochemistry"
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Oral Presentation

Asparagine-rich metastatic niches drive prostate cancer organotropism by enabling translational rewiring toward N-glycosylated proteins

Erica Pranzini[1#], Luigi Ippolito[1], Marta Iozzo[1], Simone Romagnoli[1], Greta Bertoli[1], Giacomo Venditti[1], Caterina Grillo[1], Matteo Lulli[1], Alice Santi[1], Giuseppina Comito[1], Marina La Chimia[3], Simone Polvani[1], Tiziano Lottini[2], Matteo Benelli[1], Domenica Scumaci[3], Elisa Giannoni[1*] Chiarugi Paola[1*]

[1]Dpt. of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence (Italy)
[2]Dpt. of Experimental and Clinical Medicine, University of Florence (Italy)
[3]Dpt. of Experimental and Clinical Medicine; School of Medicine Magna Græcia University of Catanzaro (Italy)

* These authors equally contributed
# e-mail: erica.pranzini@unifi.it

Background: Metastatic disease is the leading cause of mortality in prostate cancer (PC), with bone as the preferential site of dissemination and the lung frequently affected secondarily. Metabolic interactions between disseminated tumor cells (DTCs), tissue-specific microenvironments, and local metabolites play a key role in metastatic organotropism.

Aim: This study aims to identify metabolic determinants driving preferential PC dissemination to bone and lung and understand how this influence early metastatic colonization.

Results: We identify asparagine (Asn) as a niche-specific metabolite enriched in bone and lung microenvironments that supports PC metastatic colonization. Dietary Asn restriction selectively impairs bone and lung metastases *in vivo* without affecting dissemination to other organs. Mechanistically, DTCs colonizing Asn-rich niches exhibit reduced asparagine synthetase (ASNS) expression and become dependent on extracellular Asn, which activates an mTORC1-dependent translational program. Increased Asn availability specifically promotes the synthesis of Asn-rich proteins containing N-glycosylation motifs (Asn-X-Ser/Thr), enhancing global protein N-glycosylation. This adaptation promotes homotypic cell-cell interactions and facilitates adhesion to bone- and lung- specific ECM as well as to osteoblasts and alveolar cells. Among the induced N-glycosylated proteins, CD44 emerges as a key effector. Importantly, extracellular Asn depletion, inhibition of N-glycosylation, or CD44 silencing abolishes the Asn-driven metastatic advantage. Finally, Asn exposure increases PC cell resistance to activated $CD8^+$ T-cell cytotoxicity, an effect reversed by inhibition of N-glycosylation.

Conclusions: These results identify Asn as a critical metabolic determinant of PC bone and lung colonization through glycosylation-dependent mechanisms, highlighting a shared bone-lung metabolic vulnerability and suggesting therapeutic strategies targeting early metastatic colonization.

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6th Workshop of the SIB group "Tumor Biochemistry"
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Oral Presentation

PARP12 drives estrogen signaling, chromatin dynamics and therapeutic vulnerability in ER+ breast cancer

Ludovica Serpieri[1*], Maria Azzurra Caricasulo[1*], Nadia Badolati[1] and Giovanna Grimaldi[1]

[1]Institute of Endotypes in Oncology, Metabolism and Immunology (IEOMI), National Research Council (CNR), Naples, Italy.

*Equal contribution

Background: ADP-ribosylation is a post-translational modification mediated by enzymes of the PARP family, which transfer ADP-ribose units to downstream targets, thereby regulating key cellular processes ranging from cell cycle progression to stress response and immunity. PARP12 is a mono-ADP-ribosyltransferase known for its roles in antiviral activity and intracellular trafficking; more recently, it has emerged as a regulator of oncogenic signaling in estrogen receptor-positive (ER^+) breast cancer. We previously demonstrated that PARP12 mono-ADP-ribosylates AKT, sustaining ER^+ cell survival, whereas its depletion induces apoptosis and DNA damage.

Aim: To investigate the role of PARP12 in ER^+ breast cancer, focusing on ERα signaling, chromatin remodeling, and endocrine resistance.

Results: RNA-seq analysis following PARP12 silencing in MCF7 cells revealed a significant downregulation of ERα target genes. Accordingly, PARP12 depletion markedly reduced the transcriptionally active ERα phosphorylated at Ser118 and strongly impaired its nuclear translocation, suppressing estrogen-responsive transcription in both MCF7 and T47D cells. To assess whether PARP12 affected AKT- mediated ERα phosphorylation at Ser167, PARP12 silencing was compared with the AKT inhibitor capivasertib. Our data showed that PARP12 regulation of ERα signaling occurs independently of AKT, suggesting a parallel survival mechanism. In addition, PARP12 loss reduced H3K27trimethylation, supporting its role in chromatin-mediated transcriptional regulation.Notably, hormone deprivation decreased PARP12 expression, while estradiol restored its levels in both cell lines, pointing to a cross-regulation between PARP12 and ERα signaling. Consistently, PARP12 expression was upregulated in tamoxifen-resistant MCF7 cells and in patients who developed endocrine therapy resistance. Ongoing studies combining clinically relevant breast cancer therapies with PARP12-targeting siRNA aim to determine whether PARP12 sensitizes cells to pharmacological treatments.

Conclusions: PARP12 acts as a key regulator of ERα signaling and chromatin dynamics in ER+ breast cancer. Its AKT-independent control of ERα activity and H3K27me3 status contributes to tumor survival and endocrine resistance, suggesting that combining PARP12 targeting with standard therapies, or as a dual-pathway blockade with AKT inhibitors, represents a promising therapeutic strategy.

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6th Workshop of the SIB group "Tumor Biochemistry"
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Oral Presentation

GATA-1S induces drug resistance in myeloid leukemia cells by targeting sphingolipid metabolism

Silvia Trombetti[1], Raffaele Sessa[1], Najoua Chhoud[1,2], Pasquale Cirillo[1,3], and Michela Grosso[1]

[1]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; Silvia.trombetti@unina.it, Raffaele.sessa@unina.it, michela.grosso@unina.it
[2]Faculté de Sciences et Techniques De Mohammedia, Universite Hassan II de Casablanca, Casablanca, Morocco; najoua.chhoud1999@gmail.com
[3]Department of Physics "E. Pancini", University of Naples Federico II, Naples, Italy; cirillo.pasquale.1999@gmail.com

Background: Leukemia is a hematological malignancy characterized by the uncontrolled proliferation of abnormal cells due to bone marrow deregulation. The transcription factor GATA-1 is a master regulator of hematopoiesis, acting through its two isoforms: GATA-1 full-length (GATA- 1_{FL}) and GATA-1 short (GATA-1_S). While a balanced GATA-1_{FL}/GATA-1_S ratio sustains normal cell differentiation, the aberrant overexpression of GATA-1_S exerts a potent pro-leukemic effect, promoting cell survival and chemoresistance.

Aim: This study aimed to elucidate the metabolic alterations associated with GATA-1_S overexpression in myeloid leukemia. Specifically, we investigated how GATA-1 isoforms differentially modulate sphingolipid metabolism and the ceramide/sphingosine-1-phosphate (S1P) rheostat, exploring their impact on cell proliferation, ferroptosis resistance, and drug sensitivity.

Results: Lipidomic analysis on K562 myeloid leukemia cells overexpressing GATA-1_S revealed a significant reduction in total ceramide content, alongside alterations in fatty acid composition and PUFA levels that confer resistance to ferroptosis. This phenotype correlates with a strategic transcriptional remodeling of key sphingolipid enzymes. Compared to GATA-1_{FL} cells, GATA-1_S-overexpressing cells displayed upregulation of ceramidases and downregulation of specific ceramide synthases (CERS1, CERS2). Concurrently, we detected increased levels of CERK1 and SPHK1, leading to the accumulation of the pro-survival signaling molecules ceramide-1-phosphate and S1P. Notably, SPHK1 knockdown successfully impaired cell proliferation and restored sensitivity to cisplatin-induced apoptosis, effectively counteracting GATA-1_S- mediated chemoresistance.

Conclusions: Our findings demonstrate that GATA-1_S expression shifts the ceramide/S1P rheostat toward an anti-apoptotic phenotype. This sphingolipid dysregulation represents a critical metabolic hallmark of GATA-1_S-driven leukemia, highlighting SPHK1 and related pathways as promising therapeutic targets in AML.

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6th Workshop of the SIB group "Tumor Biochemistry"
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Selected Flash Talk - Poster 01

Formyl-peptide receptor 2 stimulation rewires branched-chain amino acid metabolism in glioblastoma cells

Chiara Brignola[1]*, Myrhiam Cassese[1], Stefano Marrone[1], Rosario Ammendola[1], Teresa Esposito[1], Fabio Cattaneo[1]

[1]Department of Molecular Medicine and Medical Biotechnology, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy; chiara.brignola@unina.it

Background: Formyl-peptide receptor 2 (FPR2) is a G protein-coupled receptor whose activation triggers either pro- or anti-inflammatory responses, regulating, in turn, several biological processes. Among them, accumulating evidence indicates that FPR2 stimulation can activate downstream signaling cascades that influence metabolic adaptation of cancer cells, including glucose, glutamine, and pyrimidine nucleotide pathways. Among the metabolic alterations occurring in malignant cells, reprogramming of branched-chain amino acids (BCAAs) metabolism represents a key driver of cancer growth and progression. Tumor cells frequently develop a BCAAs "addiction", essential for satisfying their increased demand for energy, nitrogen, and carbon skeletons required for rapid proliferation.

Aim: Starting from this, the aim of this study was to investigate BCAAs uptake and their catabolic flux mediated by FPR2 activation in U-87 MG glioblastoma (GBM) cells.

Results: We employed gas chromatography-mass spectrometry (GC–MS)-based metabolomics for the analysis of intracellular metabolites following stimulation of Annexin A1 (ANXA1), an endogenous FPR2 agonist. GC–MS analysis revealed altered BCAAs (leucine, isoleucine, and valine) levels in ANXA1-stimulated cells, accompanied by an upregulation of L-type AA transporter 1 (LAT1), responsible for BCAAs cellular import. Moreover, ANXA1 treatment promoted BCAAs catabolic flux by increasing their mitochondrial uptake through SLC25A44 transporter, as well as by dysregulating the expression and phosphorylation levels of key catabolic enzymes. Of interest, we also found increased levels of BCAA-derived hydroxyacid intermediates, further supporting an enhanced BCAAs turnover.

Conclusions: This study reveals an unrecognized link between FPR2 signaling and BCAA–dependent metabolic adaptation, thus highlighting a potential metabolic vulnerability of GBM cells.

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6th Workshop of the SIB group "Tumor Biochemistry"
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Selected Flash Talk - Poster 02

Acute Myeloid Leukemia: a key role of DGKα and DGKζ in cell viability

Elisa Gorla[1,2], Marco Cristiano Cartella[1,2], Ginevra Lovati[1,2], Edoardo Borghetti[1,2], Luisa Racca[1,2], Valeria Malacarne[1,3], Andrea Graziani[1,3], Alberto Massarotti[4], Davide Corà[1,2], Marcello Manfredi[1,2], Gianluca Baldanzi[1,2]

[1]Department of Translational Medicine, University of Piemonte Orientale, 28100 Novara, Italy; elisa.gorla@uniupo.it
[2]Center for Translational Research on Allergic and Autoimmune Diseases (CAAD), University of Piemonte Orientale, 28100 Novara, Italy;
[3]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center "Guido Tarone", University of Torino, 10126 Torino, Italy;
[4]Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara 28100, Italy.

Background and aims: Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by arrested myeloid differentiation, which is in part controlled by diacylglycerol kinases (DGKs). Human DGKs are a family of ten enzymes that phosphorylate diacylglycerol into phosphatidic acid modulating cell-signaling. Several DGK isoforms are highly expressed in AML. Indeed, hematopoiesis and AML development are in part controlled by either DGKα or DGKζ that are proposed as targets for a novel therapeutical approach [(1,2,3)].

Methods: To evaluate their relevance in cellular models, we pharmacologically inhibited or silenced either DGKα or DGKζ and evaluated their role by measuring cell viability and apoptosis/necrosis rate in a panel of AML cell lines.

Results: Each AML cell line resulted differently impacted by DGKα and DGKζ pharmacological inhibition or silencing. They were sensible to the DGKα specific inhibitor ritanserin in the μM range (THP1 > HL-60 > HEL > K562), exhibiting an increase in apoptosis. Even DGKζ-IN-4 andBAY2965501 (DGKζ specific inhibitors) decreased cell viability in the same range (HL-60 > THP-1 > HEL > K562) incrementing apoptosis but also necrosis. Concerning isoform specific knockdown, HEL cells were negatively affected by DGKα silencing with reduced cell viability and increased apoptosis, whereas DGKζ silencing did not exert any effect. Conversely, HL-60 cells viability decreased by apoptosis upon DGKζ silencing but unaffected by DGKα silencing. Finally, K562 and THP-1 resulted poorly sensitive to the knockdown of both isoforms.

Conclusion: These data indicate that DGKs are putative therapeutical targets for AML patients playing a relevant and isoform-specific role. Moreover, the expression level of each isoform is not a predictor of responsiveness to isoform specific inhibitors

Selected Flash Talk - Poster 03

Metabolic remodeling during NSCLC spheroids maturation: a comparative 2D/3D study

Monica Infantino[1], Rossella Mosca[1], Martina Avanatti[1], Gemma Sardelli[1], Francesca Felice[1], Antonella Del Corso[1], Simone Allegrini[1], Francesco Balestri[1], Mario Cappiello[1], Giovanni Signore[1] and Roberta Moschini[1]

[1]Department of Biology, Biochemistry Unit, University of Pisa, via S. Zeno 51, 56127 Pisa, Italy; monica.infantino@phd.unipi.it.

Background: Lung cancer remains a leading cause of cancer-related mortality worldwide, highlighting the need for physiologically relevant experimental models. Compared with conventional two-dimensional (2D) cultures, three-dimensional (3D) spheroid models better recapitulate tumor architecture, hypoxia, metabolic gradients and cell-cell interactions, representing a valuable platform for investigating tumor microenvironment-associated adaptations.

Aim: This study aims to characterize morphological and metabolic adaptations occurring during spheroid maturation in two non-small cell lung cancer (NSCLC) models, A549 and $KRAS^{G12C}$-mutant NCI-H358, focusing on hypoxia pathways, lactate metabolism and lipid remodeling.

Results: Preliminary analyses on A549 spheroids revealed progressive morphological compaction from day 3 to day 7, with reduced area and diameter and increased circularity and solidity, indicating structural organization during spheroid maturation. Alamar Blue assays showed time-dependent metabolic changes during 3D growth. In contrast, NCI- H358 spheroids displayed earlier compaction and relatively stable metabolic activity over time, suggesting distinct adaptation dynamics between NSCLC models. Ongoing analyses compare 2D monolayer cultures and 3D spheroids at different maturation stages (day 4 vs day 6), focusing on HIF1α expression, lactate quantification and time-course lipidomic profiling (days 3–7) to identify dynamic lipid remodeling and 2D/3D-specific metabolic signatures.

Conclusion: These preliminary findings support the establishment of reproducible, dynamic and metabolically active 3D NSCLC models reproducing key features of the tumor microenvironment. By integrating morphological, metabolic, and lipidomic readouts, this platform may help identify adaptive metabolic changes and provide a basis for investigating tumor-derived signals and novel therapeutic vulnerabilities.

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Selected Flash Talk - Poster 04

Uncovering the role of the secreted protein C10orf118 in breast cancer cells-derived exosomes

Varvara–Christina Siagka[1], Paola Moretto[1], Alberto Passi[1], Davide Vigetti[1], Manuela Viola[1], Evgenia Karousou[1]

[1]Department of Medicine and Surgery, University of Insubria, Via J.H. Dunant 5, 21100 Varese, Italy, christina.siagka@gmail.com; paola.moretto@uninsubria.it; alberto.passi@uninsubria.it; Davide.Vigetti@uninsubria.it; manuela.viola@uninsubria.it; jenny.karousou@uninsubria.it

Background: Exosomes are nanosized extracellular vesicles that guide cells' intercellular communications, in normal and pathological conditions. Recently, we explored the presence of the C10orf118 protein in normal fibroblasts and breast cancer (BC) cells with different estrogen receptor (ER) status, metastatic potential, and aggressiveness. Additionally, we demonstrated the implication of this protein on the hyaluronan (HA) metabolism in co-cultures of normal fibroblasts with BC cells [1].

Aim: The present study assesses whether C10orf118 protein is secreted within the exosomes of three BC cell lines: MCF-7 (ER+, Low metastatic), MDA-MB-231 and Hs578T (ER-, High metastatic).

Results: Our results indicate that C10orf118 has higher expression in MCF-7 cells and is detected both in Golgi apparatus and exosomes. Moreover, exosomes' characterization suggests that the exosomes derived from both MDA-MB-231 and MCF-7 cells are divided into two subpopulations but of different morphology and size.

Conclusions: Our findings propose that C10orf118, due to its high molecular weight, utilizes exosomes as vesicular support to secrete in the extracellular matrix. Further studies center on the treatment of normal fibroblasts with exosomes, using gene silencing technique, to decipher the role of C10orf118 in the exosomes' stromal cells functions through changes in HA metabolism.

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[1] Caon I. et al. (2021) Cancers 13, 1105

Selected Flash Talk - Poster 05

Extracellular NAMPT and NNMT characterize Braf inhibitor–resistance in melanoma cells, with preliminary evidence of nuclear localization

Beatrice Ghezzi[1*], Irene Fiorilla[1*], Ágata Carreira[2*], Francesco Recco[1,3], Leonardo Sorci[4], Lidia Avalle[1], Alessia Ponzano[1], Francesca Mazzola[4], Alberto Maria Todesco[1], Nicoletta Tommasi[1], Massimiliano Gasparrini[4], Vito Giuseppe D'Agostino[2], Flavio Mignone[3], Alessandro Provenzani[2], Valentina Audrito[1]

[1]Department of Science and Technological Innovation, University of Eastern Piedmont, Alessandria, Italy.
[2]Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy.
[3]SmartSeq s.r.l., Alessandria, Italy.
[4]Department of Science and Engineering of Matter, Environment and Urban Planning, Polytechnic University of Marche, Ancona, Italy.

*These authors contributed equally to this work

Background: Resistance to BRAF inhibitors (BRAFi) in metastatic melanoma (MM) involves complex tumor-intrinsic and tumor- microenvironment–mediated mechanisms. Alterations in nicotinamide (NAM) metabolism are emerging as key contributors to therapy resistance, although their functional role remains only partially understood. Here, we investigated the role of the NAM rate-limiting enzymes nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide N- methyltransferase (NNMT), at both intracellular and extracellular levels, in BRAFi-resistant melanoma models.

Aims: To investigate the involvement of NAMPT and NNMT at both intracellular and extracellular levels, including their release as soluble proteins and their association with extracellular vesicles (EVs), in BRAFi- resistant melanoma, and to explore their subcellular localization and nuclear trafficking in resistant cells.

Results: NAMPT, already known to be upregulated in MM, and NNMT, not previously described in this context, were significantly overexpressed in BRAFi-resistant MM cells. Both enzymes were detected in the culture supernatants of resistant cell lines and were identified, for the first time, in EVs released from resistant MM cells. Analysis of the TCGA melanoma cohort revealed a positive correlation between NAMPT and NNMT expression and a significant association between NNMT levels and pro-inflammatory, cell migration, and chemokine-related pathways in BRAF- mutated patients, who displayed higher NNMT expression than BRAF wild-type cases, suggesting a link between BRAF signalling and NNMT upregulation. In addition, we found that both NAMPT and NNMT are moreabundant in the nuclei of BRAFi-resistant cells and are enriched in the chromatin-associated fraction of resistant cells. Focusing on NAMPT, we show that its nuclear localization is cell-cycle dependent, with a marked increase during the G2 phase. Moreover, NAMPT undergoes nuclear translocation in response to DNA damage induced by etoposide. Inhibition of exportin-1 resulted in a pronounced nuclear accumulation of NAMPT, indicating that its nucleo-cytoplasmic trafficking is regulated by an activeexport mechanism.

Conclusion: Our results reveal a dual intracellular and extracellular role for NAMPT and NNMT in BRAFi resistance in MM and uncover an additional nuclear and chromatin-associated pool of NAMPT that is dynamically regulated by cell-cycle progression and DNA damage. The presence of NAM-metabolizing enzymes in the secretome and in EVs, together with their nuclear redistribution in resistant cells, highlights a multifaceted contribution of NAM metabolism to tumor cell plasticity and tumor microenvironment reprogramming, suggesting that targeting this pathway may represent a promising strategy to overcome resistance to targeted therapies.

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6th Workshop of the SIB group "Tumor Biochemistry"
Bologna, July 9-10th, 2026

Selected Flash Talk - Poster 06

ER-mitochondria contact sites modulate melanoma progression and chemoresistance

Michela Rossini[1], Elisabetta Zaltron[1], Ilaria Celotti[1], Sabrina Toffanin[1], Alessia Ruzza[1], Federica Vianello[1], Matteo Scavezzon[1], Angelo Velle[1], Aurora Madiolo[1], Lara Iocco[1], Annalisa Paronetto[1], Filippo Severin[1], Tito Calì[2], Marisa Brini[3], Riccardo Filadi[2], Paola Pizzo[2], Chiara Romualdi[1], Gyorgy Hajnoczky[4], Luigi Leanza[1]

[1]Department of Biology, University of Padua, michela.rossini@phd.unipd.it, luigi.leanza@unipd.it
[2]Department of Biomedical Sciences, University of Padua
[3]Department of Pharmaceutical and Pharmacological Sciences, University of Padua
[4]MitoCare Center for Mitochondrial Imaging Research and Diagnostics, Department of Pathology and Genomic Medicine, Thomas Jefferson University, Philadelphia, USA.

Background: Skin cutaneous melanoma is a highly aggressive malignancy characterized by a strong metastatic potential and frequent resistance to targeted therapies, thus highlighting the importance of finding novel therapeutical targets to exploit. Among those has emerged Glucose Regulated Protein 75 (GRP75), which is enriched in cancer cells and correlates with poor prognosis and therapy resistance in multiple cancer types. Importantly, GRP75 is a key regulator of endoplasmic reticulum (ER)-mitochondria contact sites (MERCs).

Aim: This work investigates the contribution of ER–mitochondria contacts to melanoma tumorigenesis and chemoresistance, with the final aim to assess the potential targeting of MERCs to ameliorate patient clinical outcome.

Results: GRP75 was stably silenced in both murine and human melanoma cell lines. Functional analyses of melanoma proliferation and migration *in vitro* and *in vivo*, together with the evaluation of mitochondrial activity, pharmacological response and ER-mitochondria contact site dynamics were assessed. Disruption of ER–mitochondria contact sites caused a significant impairment of melanoma proliferation and migration *in vitro* and *in vivo*, alterations in mitochondrial metabolism, and drug sensitivity.

Conclusions: Our data indicate that ER–mitochondria contact regulation tunes tumorigenesis with MERCs required to sustain melanoma aggressiveness and chemoresistance. Indeed, mitochondria-ER tethering may be beneficial against cancer by altering the cellular signaling, and in turn sensitizing tumor cells to chemotherapeutic treatment.

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Selected Flash Talk - Poster 07

uL3 Downregulation drives Colorectal Cancer Stemness and Chemoresistance: Targeting with Vitamin C in 2D, 3D and CAM Models

Zeudi Petrone[1], Annalisa Pecoraro[1], Camilla Danisi[1], Giulia Russo[1] and Annapina Russo[1]

[1]Dept. of Pharmacy, University of Naples "Federico II", Via Domenico Montesano, 49, 80131 Naples, Italy.; zeudi.petrone@unina.it; camilla.danisi@unina.it; annalisa.pecoraro@unina.it; giulia.russo@unina.it; annapina.russo@unina.it

Background: Cancer stem cells (CSCs) sustain colorectal cancer (CRC) progression, dissemination, and therapy resistance. Ribosomal protein dysregulation is emerging as a regulator of CSC properties[1]. Consistently, our previous studies have shown that low uL3 expression correlates with chemoresistance and a more aggressive, pro-angiogenic, and metastatic phenotype[2]. Based on the reported anti-CSC and chemosensitizing activity of vitamin C[3], we investigated its potential to counteract uL3-associated aggressiveness.

Aim: To investigate whether uL3 downregulation promotes CSC-associated features in CRC cells and to evaluate the ability of vitamin C to counteract this phenotype and sensitize uL3-deficient cells to 5-fluorouracil (5-FU) in 2D, 3D, and in vivo models.

Results: uL3 downregulation in HCT 116p53-/- cells promoted a CSC-like phenotype, associated with differential modulation of EpCAM, CD133 and CD44 and with post-transcriptional mechanisms involving mRNA stability and translational control. This phenotype was confirmed in 3D cultures, in which low- uL3 cells exhibited enhanced spheroid formation and increased CSC marker positivity. Vitamin C reduced cell viability and stem-like features in 2D, impaired spheroid growth/viability in 3D and sensitized uL3-deficient cells to 5-FU. In the CAM model, low-uL3 cells generated larger, more vascularized tumors with increased dissemination to embryo organs. Vitamin C, alone or combined with 5- FU, limited tumor growth, vascularization and metastatic spread.

Conclusions: uL3 downregulation promotes CSC-associated traits and contributes to an aggressive, 5-FU-resistant CRC phenotype. Vitamin C counteracts these features across 2D, 3D and in vivo models and improves 5-FU response, supporting its potential use as an adjuvant strategy in CRC with uL3 dysregulation.

Reference:

1. Samanta P. et al. Ribosome biogenesis and ribosomal proteins in cancer stem cells: a new therapeutic prospect. *Molecular Biology Reports*, 2024. doi:10.1007/s11033-024-09963-y.
2. Brignola C. et al. uL3 Regulates Redox Metabolism and Ferroptosis Sensitivity of p53-Deleted Colorectal Cancer Cells. *Antioxidants*, 2024. doi:10.3390/antiox13070757.
3. Lee Y. Role of Vitamin C in Targeting Cancer Stem Cells and Cellular Plasticity. *Cancers*, 2023. doi:10.3390/cancers15235657.

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Poster 08

Multi-omics profiling of different types of pituitary adenomas

M'hammed Aguennouz[1], Polito Francesca[1], Laura Bianchi[2], Irene Gasparo[1], Laura Licitri[1], Vincenzo Macaione[1], Salvatore Cannavò[3].

[1]Department of Clinical and Experimental Medicine, University of Messina, Italy; email:aguenoz@unime.it
[2]Laboratory of Functional Proteomics, Department of Life Sciences, University of Siena, Italy;
[3]Department of Adult and Developmental Human Pathology University of Messina, Italy

Background: Pituitary adenomas are among the most prevalent neoplasms of the endocrine system and, although generally benign, they may cause significant hormonal dysregulation and clinical complications. DNA methylation, which affects gene expression profiles, is one of the major epigenetic mechanisms involved in apoptosis, cell proliferation, and cellular transformation. The remarkable biological heterogeneity of pituitary adenomas makes both diagnosis and therapeutic management particularly challenging, highlighting the need to identify novel biomarkers and further elucidate the underlying molecular mechanisms.

Aim: The aim of this study was to characterize the genome-wide DNA methylation and proteomic profiles of different pituitary adenoma subtypes and normal pituitary tissue.

Results: The analysis identified 178 differentially methylated target regions ($P \leq 0.05$). Two regions were found to be significantly hypomethylated in growth hormone-secreting adenomas (GHs) compared with non-functioning pituitary adenomas (NFPAs). NFPAs exhibited a distinct global methylation profile compared with GHs. Aberrant DNA methylation appeared to contribute to the dysregulation of cancer-related pathways, including those involving **ERN1**, which contributes to the basal production of pro-tumorigenic factors, and **ASXL1**. Proteomic analysis revealed alterations affecting metabolic processes, cell cycle regulation, and cytoskeletal reorganization. Pathway analysis identified **ALDOA** and **UCHL1** among the central hubs, both of which are known to be involved in various neoplasms and may play important roles in the biology of pituitary adenomas. Furthermore, the altered abundance of **SOD1** and **SOD2** suggests the presence of oxidative stress, as well as disruptions in cellular proliferation and the maintenance of cellular integrity.

Conclusions: In conclusion, the integration of proteomic and DNA methylation analyses revealed specific molecular alterations associated with pituitary adenomas. These findings provide new insights into the biological characterization of these tumors and may represent a starting point for the identification of novel biomarkers with potential diagnostic and prognostic value.

Poster 09

Development of a 3D Tricellular Spheroid Model to Investigate Macrophage-Driven Drug Resistance and Microenvironmental Interactions in Osteosarcoma

Lorenzo Anconelli[1], Martina Rossi[1], Aurora Mioli[1], Francesca Gorini[2], Gloria Ravegnini[2], Alessandra Borriello[3], Melania Barbagallo[3], Francesca Rossi[1], Concettina Cappadone[1], Emil Malucelli[1] and Stefano Iotti[1]

[1]FaBit Department, University of Bologna, Bologna
[2]FaBit Department, University of Bologna, Bologna
[3]UOC Anatomia Patologica, DIMEC Department, University of Bologna, Bologna

Background: Osteosarcoma (OS) is an aggressive bone malignancy characterized by a highly complex tumor microenvironment (OS-TME). Within this niche, tumor-associated macrophages, particularly those exhibiting an M2 like phenotype, play a critical role in driving tumor progression, metabolic rewiring, and chemoresistance.

Aim: Aim of this project was to develop and validate a 3D tricellular spheroid platform composed of OS cell lines (143B or MG63), fibroblasts, and THP1 monocytes. This model was designed to track tumor-driven monocyte recruitment, monitor their in situ differentiation and polarization, and investigate specific mechanisms through which macrophages promote drug resistance and modulate metabolic responses within the OS-TME.

Results: We successfully established stable 3D tricellular spheroids. Immunohistochemistry analyses confirmed that THP-1 monocytes differentiated into macrophages, and acquired a pro-tumorigenic M2-like phenotype under the tumor-conditioned microenvinroment cues. To evaluate the platform's utility in drug resistance assays, spheroids were treated with Lichocalcon-A (an interesting compound known for its antitumor activity) for 72 hours. Interestingly, we observed an increase in cell viability in presence of THP1. RT-qPCR analysis revealed significant upregulation of the MDR1 gene, indicating a macrophage-mediated protective effect against drug-induced cytotoxicity.

Conclusions: We developed a robust and physiologically relevant 3D tricellular platform that recapitulates key cellular interact6ion within the OS microenvironment. Our findings underscore the pivotal role of M2-like macrophages in modulating tumor cell survival and promoting chemoresistance, at list in part through a MDR1 upregulation. The results obtained by this platform provide a deeper knowledge of the metabolic and microenvironmental impacts of TAMs, serving as a valuable tool for identifying novel therapeutic targets in OS.

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Poster 10

Hyperinsulinemia induces a redox-adapted state in MCF-7 breast cancer cells

Martina Avanatti[1], Rossella Mosca[1], Monica Infantino[1], Gemma Sardelli[1], Francesca Felice[1], Giovanni Signore[1], Antonella Del Corso[1], Cappiello Mario[1], Allegrini Simone[1] Francesco Balestri[1] and Roberta Moschini[1]

[1]University of Pisa, Department of Biology, Biochemistry Unit; martina.avanatti@phd.unipi.it

Background: Hyperinsulinemia is associated with an increased risk of breast cancer (BC) and poor prognosis, although the underlying molecular mechanisms remain partially understood Hyperinsulinemia may promote cancer progression activating PI3K/AKT/mTOR and RAS/RAF/MAPK signaling pathways. Chronic hyperinsulinemia frequently coexists with systemic inflammation, dyslipidemia, and hypertension. Among inflammatory mediators, IL-6 is overexpressed in aggressive BC and contributes to proliferation, angiogenesis, invasion, and resistance to apoptosis through STAT3 signaling.

Aim: To investigate whether chronic insulin exposure induces a metabolic and redox-adapted state in BC cells primed for inflammatory remodeling.

Results: MCF-7 cells were exposed to chronic insulin treatment (0.1 µg/mL, ≥72 h). Insulin markedly increased AKT phosphorylation and intracellular ROS levels. Treatment was also associated with selective upregulation of the redox enzyme AKR1C3, whereas AKR1B10 and thioredoxin-1 remained unchanged. Despite evidence of redox adaptation, insulin exposure did not induce proliferation-, invasion-, or inflammatory markers, including MMP2, ADAMTS, NLRP3, and iNOS. Notably, increasing insulin concentrations progressively enhanced AKT phosphorylation, while AKR1B10 upregulation was observed under maximal insulin stimulation (1.0 µg/mL). Ongoing studies are evaluating whether insulin-induced redox rewiring, alone or with IL-6, modulates therapy response.

Conclusions: Chronic insulin exposure induces selective metabolic and redox rewiring in BC cells without directly promoting aggressive phenotypes. These findings support a multi-hit model of BC progression in metabolic disease, in which hyperinsulinemia may act as a permissive priming factor potentially predisposing tumor cells to respond more strongly to additional pro-tumorogenic stimuli. Among these, inflammatory signals such as IL-6 may promote the transition toward tumor progression and therapy resistance.

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Poster 11

Metabolomics approach reveals drug-induced metabolic dysregulations in glioblastoma cells

Palmina Bagnara[1], Carmela Dello Russo[1], Carolina Manzo[1], Federica Renza[1], Luisa Gesualdi[2], Claudia Abbruzzese[2], Marco G. Paggi[2], Alfredo Budillon[3], Elena Di Gennaro[1], and Susan Costantini[1]

[1]Experimental Pharmacology Unit, Laboratori di Mercogliano, Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale; palmina.bagnara@istitutotumori.na.it; carmela.dellorusso@istitutotumori.na.it; s.costantini@istitutotumori.na.it;
[2]Cellular Networks and Molecular Therapeutic Targets, Proteomics Unit, IRCCS - Regina Elena National Cancer Institute, Rome;
[3]Scientific Directorate, Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale, Napoli

Background: Glioblastoma (GBM) is characterized by rapid growth, high molecular heterogeneity, and invasiveness. Current standard-of-care for GBM includes surgical resection, followed by radiotherapy plus concomitant and adjuvant chemotherapy with temozolomide. However, patients almost invariably succumb due to therapy resistance and disease recurrences. Therefore, novel therapies for GBM are urgently needed to improve patient survival. In the last years, we are focusing on targeting of PKM2, a key metabolic gatekeeper which drives aerobic glycolysis in its cytoplasmic tetrameric form whereas acts as an oncogenic co-activator that directly regulates gene transcription in its nuclear dimeric state.

Aim: Since omics technologies, such as metabolomics, can generate data useful to elucidate the complex molecular mechanisms driving GBM progression, and to identify new diagnostic and prognostic biomarkers, we performed metabolomics studies by Liquid Chromatography-Mass Spectrometry (LC/MS) technique on GBM cell lines.

Results: Untargeted metabolomics profiling has been evaluated on four GBM cell line treated with three drugs, able to modulate the PKM2 tetramer/dimer ratio selected by a computational screening on a drug library, compared to untreated cells. Polar phases of cell pellets were extracted by chemical mixture based on water, methanol and chloroform. We identified the metabolites modulated from each treatment in four GBM cells evidencing that three compounds modulate a distinct number of metabolites, characterized by both overlapping and drug-specific alterations.Since our interest regards also the metabolic pathways commonly regulated by the three treatments, we performed enriched pathway analysis.

Conclusions: Metabolomic profiling of treated GBM cells revealed drug-induced dysregulations in metabolic pathways, providing a foundation for advanced clinical strategies.

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Poster 12

Temporal Metabolic Vulnerability of Glioblastoma Cells Induced by Focused Sonoporation

Francesco Bellia[1], Konstantinos Partsinevelos[1], Sofia Paola Lombardo[1], Tatiana Zuppelli[1], Jessica Ferrigno[1], Giuseppe Niccolò Conti[1], Emanuela Tropea[1], Lucia Longhitano[1], Giovanni Li Volti[1], Daniele Tibullo[1] and Angela Maria Amorini[1]

[1]Department of Biomedical and Biotechnological Sciences, University of Catania; francesco.bellia@unict.it

Background: Glioblastoma (GBM) is a highly aggressive brain tumor with limited therapeutic options. Focused sonoporation is an emerging technique that uses ultrasound waves to transiently increase cell membrane permeability, improving drug delivery with high spatial and temporal control. Although sonoporation involves short-term cellular stress, its downstream metabolic consequences on cancer cells require deeper investigation.

Aim: This study investigated the post-sonoporation metabolic kinetics in A172 glioblastoma cells to identify a metabolic temporal window that could be exploited to enhance therapeutic efficacy.

Results: Metabolomic analysis of 70 compounds revealed a severe metabolic derangement in A172 cells 3 hours post-treatment. This was characterized by a decline in total nucleotide triphosphates (NTPs), decreased pyruvate, and a lower NAD+/NADH ratio. Depletion of GSH and NADPH indicated a weakened antioxidant defense system, while a significant reduction in UDP-sugars pointed to compromised protein glycosylation. This metabolic collapse persisted at 6 hours, followed by a weak recovery between 12 and 24 hours. Notably, healthy microglial HMC3 cells subjected to the same treatment showed a completely opposite trend (an increase) in energetic and UDP-sugar metabolites at the 3-hour mark.

Conclusions: Focused sonoporation triggers a selective and transient metabolic collapse in A172 glioblastoma cells, peaking at 3 hours post-treatment. This response is not observed in non-cancerous HMC3 cells, suggesting a translational effect that creates a specific window of vulnerability. These findings open new avenues to study the involved pathways and optimize timed drug delivery strategies.

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Poster 13

Prostate cancer organotropism: unraveling the role of citrate in the metabolic interplay between bone resident cells and metastatic prostate cancer cells

Greta Bertoli[1], E. Pranzini, A. Santi, L. Ippolito, M. Iozzo, S. Roccabianca, C. Grillo, G. Venditti, G. Comito, E. Giannoni, P. Chiarugi

[1]Department of Experimental, Clinical and Biomedical Sciences "Mario Serio", University of Florence, Viale Morgagni 50, 50134 Florence, Italy, g.bertoli@studenti.unisi.it

BACKGROUND AND AIMS: Patients affected by metastatic prostate cancer (PC) show a survival of 30% within 5 years from diagnosis. Clinical records show a clear metastatic organotropism of PC to bone. Multiple factors are known to drive this phenomenon, such as the interaction between metastatic cancer cells (MCCs) and the organ-specific resident cells. Specifically, MCCs can establish a metabolic interplay with resident cells by inducing them to provide functional metabolites for metastatic colonization. Based on this evidence, our aim is to analyze the metabolic interplay between PC cells and bone resident cells to identify common mechanisms sustaining PC organotropism.

RESULTS: Incubating PC cells with hFOB (human foetal osteoblasts) CM resulted in an increased lipid droplets (LDs) accumulation in two metastatic PC models, ICB and CAB (obtained by Intra Cardiac or Caudal Artery injection), but not in the parental line WT. The same effect was observed using heat-treated CM, indicating the functional contribution of heat-stable metabolites. GC-MS-based metabolomic profiling of hFOB CM identified citrate as their most secreted metabolite and its secretion was further enhanced when hFOB were pre-conditioned with Met-PC3-derived CM. Conversely, ICB and CAB metastatic cells exhibited a reduction of citrate secretion compared to WT cells, consistently with a downregulation of citrate synthase (CS) and higher expression of SLC13A5, the carrier that mediates the uptake of citrate from the extracellular microenvironment, in ICB and CAB cells compared to WT cells. Finally, treatment of Met-PC3 with exogenous citrate (0.5 μM) induced a LDs increase similar to the one obtained with heat-treated hFOM CM, suggesting citrate as a crucial mediator of lipid storage.

CONCLUSIONS: Overall, our evidence reveals that osteoblasts release high levels of citrate, which is selectively internalized by metastatic PC3 cells characterized by low CS expression. Given that extracellular citrate is known to drive cancer dissemination, this metabolic crosstalk within the bone niche likely triggers a specialized lipid rewiring. The resulting accumulation of LDs in metastatic cells may provide a critical functional advantage, ultimately fostering metastatic success in the bone microenvironment.

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Poster 14

Metabolic and Inflammatory Crosstalk in Triple-Negative Breast Cancer Brain Metastasis: The Role of IKKβ and Arginine Metabolism

Giulia Elizabeth Borsatti[1], Antonietta Marongiu[1], Serena Rinaldo[1], Francesca Cutruzzolà[1], Alessio Paone[1]

[1]Department of Biochemical Science A. Rossi Fanelli, Sapienza University of Rome;
giuliaelizabeth.borsatti@uniroma1.it, marongiu.2119848@studenti.uniroma1.it, serena.rinaldo@uniroma1.it, Francesca.cutruzzolà@uniroma1.it, alessio.paone@uniroma1.it

Background: Brain metastasis is a severe complication of triple-negative breast cancer (TNBC) with poor prognosis and limited treatment options. Emerging evidence suggests that metastatic colonization of the brain depends on coordinated inflammatory signaling and metabolic reprogramming. The IKKβ signaling pathway has been implicated in promoting metastatic progression and adaptation within the brain microenvironment, and previous studies from our group demonstrated that IKKβ signalling promotes the invasive phenotype of brain-seeking TNBC cells through regulation of pro-inflammatory cytokines and chemokines (GRO-α, GRO-β, GRO-γ, IL-8). Through functional and molecular analyses, we also identified an imbalance in IKK isoform expression, characterized by reduced IKKα and increased IKKβ levels, suggesting a shift toward a pro-metastatic inflammatory signalling program. To better define the downstream pathways controlled by IKKβ, we performed transcriptomic profiling of brain-seeking TNBC cells, which revealed increased expression of ARG2 (arginase 2). In addition, preliminary observations from our group further pointed to arginine metabolism as a potentially relevant pathway in metastatic progression.

Aim: This study aimed to define downstream pathways regulated by IKKβ in brain-seeking TNBC cells, with a focus on identifying metabolic adaptations, particularly arginine metabolism, and investigating the role of tumor astrocyte interactions and nutrient availability in the metastatic brain niche.

Results: Previous functional data confirmed that IKKβ regulates inflammatory cytokines and chemokines associated with migration and angiogenesis, as well as glutamate metabolism, indicating broad metabolic and inflammatory control. Additional observations indicated altered IKK isoform expression, characterized by decreased IKKα and increased IKKβ. Ongoing investigations show that arginine availability and inhibition of ARG2 may influence tumor growth and immune modulation. Furthermore, interactions between TNBC cells and astrocytes suggest that both cell types contribute to arginine metabolism, while tumor cells appear to actively regulate extracellular arginine levels, potentially affecting nutrient availability and metabolite involved in antitumoral defense within the metastatic niche.

Conclusion: Overall, these findings identify IKKβ as a central regulator linking inflammatory signaling and metabolic reprogramming in TNBC brain metastasis. Arginine metabolism, particularly through ARG2, emerges as a key downstream pathway that may support tumor adaptation, immune modulation, and survival in the brain microenvironment, representing a potential therapeutic vulnerability.

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Poster 15

TGS1-mediated 5' cap trimethylation regulates translation of target mRNAs in AML

Maxim Bouvet[1], Valentina Miano[2,*], Giovanna Carrà[3], Jan Lj Miljkovic[4], Vincent Paupe[4], Chak S. Yu[4], Domenico Ignoti[1], Sara Milioni[1], Daniele Viavattene[1], Anna M. Benedetti[2,♦], Shi-Lu Luan[3,#], Davide Acquarone[1], Valeria Poli[1], Paolo E. Porporato[1], Stefano Gustincich[5], Suzanne D. Turner[3,6], Maria P. Martelli[7], Alessio Menga[8], Lorenzo Brunetti[7,9], Michael P. Murphy[4,10], Luca Pandolfini[5] and Isaia Barbieri[1,2]

[1]Department of molecular biotechnology and health sciences, Molecular Biotechnology Center, University of Turin, Turin, Italy
[2]Division of cellular and molecular pathology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom
[3]San Luigi Gonzaga Hospital, Regione Gonzole 10043, Orbassano, Italy; Department of Clinical and Biological Sciences, University of Turin, Italy;
[4]MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom
[5]Central RNA Lab, Istituto Italiano di Tecnologia, Genoa, Italy
[6]Faculty of Medicine, Masaryk University, Brno, Czech Republic
[7]Department of Medicine and Surgery, Hematology, Center for Hemato-Oncological Research (CREO), University of Perugia, Perugia, Italy
[8]Department of health science, University of eastern Piedmont, Palazzo Bellini, Via Solaroli 17, Novara, Italy
[9]Department of Clinical and Molecular Sciences, Hematology, Università Politecnica delle Marche, Ancona, Italy
[10]Department of Medicine, University of Cambridge, Cambridge, United Kingdom

*Present address: Tagomics Ltd, Little Abington, Cambridge, United Kingdom
♦Present address: Cancer metabolism laboratory, The Francis Crick Institute, London, United Kingdom
#Present address: Protein & Nucleic Acid Chemistry Division, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

Background: The vast majority of primary RNA transcripts undergo extensive post-transcriptional maturation, including 5'-capping, splicing, and chemical modifications that influence RNA fate and function. Among these, 5′cap hypermethylation by Trimethylguanosine Synthase 1 (TGS1) generates 2,2,7-trimethylguanosine ($m^{2,2,7}G$), a modification known to be linked to snRNA metabolism. However, its role in mRNA regulation remains largely unexplored.

Aim: To investigate the role of TGS1 and $m^{2,2,7}G$ cap hypermethylation in the regulation of nuclear-encoded mitochondrial mRNAs, with a focus on their localization and translational control.

Results: We identified a novel function of TGS1 in promoting translation of mitochondrial mRNAs. The short TGS1 isoform localizes to both nucleus and cytoplasm, while the long isoform is cytoplasmic, with both enriched at ER–mitochondria interfaces. Transcriptomic analyses revealed enrichment of mitochondrial proteins among TGS1 targets, including one-third encoding OXPHOS components. TGS1 depletion reduced mitochondrial association of these mRNAs without affecting mitochondrial mass. APEX2 assays excluded defects in mRNA localization to the outer mitochondrial membrane (OMM), indicating a translational mechanism. Despite reduced cytoplasmic $m^{2,2,7}G$, EIF3b localization was unchanged, while CLIP-qPCR showed increased EIF3b binding to target mRNAs upon TGS1 knockdown, suggesting impaired elongation. Consistently, TGS1 is required for efficient translation at the OMM.

Conclusions: TGS1-mediated 5′-cap hypermethylation enhances translation of TCA and OXPHOS genes, sustaining mitochondrial respiration and limiting ROS accumulation. These findings uncover a previously unrecognized role for TGS1 in mitochondrial metabolism with potential implications in cancer.

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Poster 16

PON2 Silencing Enhances Cisplatin Sensitivity in Triple-Negative Breast Cancer Cells: A FTIRM Study

Roberto Campagna[1], Chiara Santoni[2], Elisabetta Giorgini[2], Veronica Pompei[3], Valentina Pozzi[3], Valentina Notarstefano[4], Giulia Orilisi[3], Monica Emanuelli[3] and Davide Sartini[3]

[1]Department for the Promotion of Human Science and Quality of Life, San Raffaele Roma University, Via di Val Cannuta, 247, 00166 Rome, Italy; roberto.campagna@uniroma5.it
[2]Department of Life and Environmental Sciences, Polytechnic University of Marche, 60131 - Ancona, Italy; c.santoni@pm.univpm.it; e.giorgini@staff.univpm.it;
[3]Department of Clinical Sciences, Polytechnic University of Marche, 60020 - Ancona, Italy; v.pompei@staff.univpm.it; v.pozzi@univpm.it; g.orilisi@pm.univpm.it; m.emanuelli@univpm.it; d.sartini@univpm.it
[4]Department of Bioscience and Agro-Food and Environmental Technology, University of Teramo, 64100 -Teramo, Italy; vnotarstefano@unite.it

Background: Triple-negative breast cancer (TNBC) is an aggressive tumor with limited treatment options and poor prognosis. Paraoxonase-2 (PON2), an oxidative stress-related enzyme, may protect tumor cells from cisplatin-induced damage.

Aim: This study assessed whether PON2 silencing could enhance cisplatin sensitivity in MDA-MB-231 TNBC cells and investigated treatment-related macromolecular changes.

Results: Cells were transfected with control pLKO.1-puro (p0) or PON2-silencing pLKO.1–647 (p647), treated with 1 μM cisplatin at different timepoints, and analyzed by MTT assay and Fourier Transform InfraRed Microspectroscopy (FTIRM). IR spectra (4000–900 cm^{-1}, 256 scans, 4cm^{-1} resolution) were acquired from ~80 micro-areas containing 2–3 cells. Spectra were pre-processed and subjected to multivariate and univariate analyses to detect cisplatin-induced macromolecular changes. FTIRM analysis, in agreement with MTT results, showed a reduction in DNA (A965), Z-DNA (A933), and RNA (A991) signals in both p0 and p647 cells, with a greater decrease in PON2-silenced cells after 24 and 48 hours of treatment, indicating reduced proliferation. Moreover, a decrease was detected in the DNA/RNA ratio (A1020/A1120) and in the dsDNA/free phosphate ratio (A965/A1085), more evident in p647 cells. In addition, lipid-related spectral changes were observed, in association with an increase in CH3 (A2957) and a concomitant reduction in CH2 (A2921), suggesting lipid chain shortening and possible cisplatin-induced plasma membrane damage, consistent with early apoptotic events.

Conclusions: These findings indicate that PON2 downregulation increases the sensitivity of MDA-MB-231 cells to cisplatin. The combined use of viability assays, FTIRM profiling, and statistical analysis allowed a more comprehensive characterization of the biochemical alterations associated with drug response, contributing to the understanding of TNBC chemoresistance mechanisms and of the functional involvement of PON2.

Funding: N.A.

Poster 17

Hyperforin Targets Mitochondrial Function and Dynamics in Dabrafenib-Resistant Melanoma Cells

Alessia Cardile, Carlotta Passarini, Raffaella Pacchiana, Adriana Celesia, Massimo Donadelli, Marta Menegazzi* and Alessandra Fiore*

Section of Biochemistry, Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Strada Le Grazie 8, Verona, 37134, Italy.

* These authors contributed equally
Presenting Author: Alessia Cardile alessia.cardile@univr.it

Background: Acquired resistance to BRAF inhibitors represents a major clinical challenge in BRAF-mutated melanoma and is frequently associated with metabolic rewiring and increased mitochondrial dependence. Targeting mitochondrial function and redox balance may represent an effective strategy to overcome drug resistance.

Aim: Therefore, we investigated whether Hyperforin (HPF) could target resistance-associated metabolic adaptations by affecting mitochondrial dynamics, bioenergetics, and oxidative stress in dabrafenib-sensitive and -resistant melanoma cell lines.

Results: Confocal microscopy revealed that HPF induced a profound remodeling of mitochondrial architecture, shifting to a fragmented phenotype. Flow cytometry analysis showed a significant increase in mitochondrial membrane potential together with elevated mitochondrial and cellular ROS post-treatment, particularly in resistant cells. Immunoblotting demonstrated that HPF rewires cellular signaling pathways, affecting cell cycle regulators (pRb, CDC25C) through the modulation of key survival pathways (STAT3, CRAF/ERK). Moreover, HPF induced apoptotic and autophagic/mitophagic markers (e.g., cPARP, LC3B, SQSTM1, PINK1) while downregulating anti-apoptotic proteins (BCL-2, MCL1, BCL-XL, Survivin). Proteins involved in mitochondrial dynamics (OPA1, DRP1, Mitofusins) were significantly affected, indicating a coordinated disruption of mitochondrial integrity, and metabolic homeostasis, especially in resistant cells. Functional bioenergetic analysis confirmed a marked impairment of mitochondrial respiration following HPF treatment, with more pronounced in dabrafenib-resistant models.

Conclusions: Hyperforin induces a coordinated collapse of mitochondrial function and cellular metabolism in melanoma cells, promoting oxidative stress and apoptotic/autophagic signaling, with enhanced susceptibility observed in dabrafenib-resistant lines. These findings highlight HPF as a promising strategy to target resistance-associated metabolic adaptations.

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Poster 18

Investigating the crosstalk between Schwann cells and macrophages in peripheral nerve sheath tumor

Maria Egle Castorina[1,2], Margherita Toffanin[1,2], Francesco Ciscato[2], Samuele Negro[1], Paolo Finotti[3], Valentina Zappulli[3], Giuseppe Persico[1], Marco Giorgio[1], Marco Fantuz[4], Alessandro Carrer[4,5], Andrea Rasola[1] and Ionica Masgras[2]

[1]Department of Biomedical Sciences, University of Padova, Padova, Italy; mariaegle.castorina@studenti.unipd.it; margherita.toffanin@studenti.unipd.it; samuele.negro@unipd.it; giuseppe.persico@unipd.it; marco.giorgio@unipd.it; andrea.rasola@unipd.it
[2]National Research Council (CNR), Institute of Neuroscience, Padova, Italy; francesco.ciscato@cnr.it; ionica.masgras@unipd.it
[3]Department of Comparative Biomedicine and Food Science, University of Padova, Italy; paolo.finotti.1@studenti.unipd.it; valentina.zappulli@unipd.it
[4]Veneto Institute of Molecular Medicine, Padova, Italy; marco.fantuz@unipd.it
[5]Department of Biology, University of Padova, Padova, Italy; alessandro.carrer@unipd.it

Background: Malignant peripheral nerve sheath tumors (MPNSTs) are rare, aggressive and incurable sarcomas. Approximately half arise in patients affected by Neurofibromatosis type 1 (NF1), an autosomal dominant multisystem disorder caused by loss-of-function mutations in neurofibromin, while the remaining cases are sporadic or radiation-induced. MPNSTs display a complex tumor microenvironment (TME), comprising neoplastic Schwann cells (SCs), fibroblasts, neurons, endothelial cells, extracellular matrix components and immune cells. Among the immune infiltrate, Tumor Associated Macrophages, identified as important players in the growth of many cancers, represent the most abundant population.

Aim: My PhD project aims to identify possible dysregulated metabolic routes in neoplastic SCs that may impinge on their crosstalk with macrophages, thereby promoting MPNSTs development.

Results: We are taking advantage of a genetically engineered mouse model in which *Nf1* and *p53* are specifically inactivated in SCs through a tamoxifen-inducible CreERT system under the control of PLP promoter. In this model a crush (*i.e.* compression) injury to the sciatic nerve triggers the development of neoplastic lesions compatible with MPNSTs in a synchronized and robust manner, approximately 30 days post-injury. We have characterized sciatic nerve specimens from Cre-positive mice and controls during the pre-tumoral phase, at both protein (immunofluorescence and immunohistochemistry) and transcriptomic level, revealing an altered transcriptomic profile together with an increased cell proliferation.

Conclusions: These differences in the injury-response may represent a pro-tumoral predisposition signature in Cre-positive mice. We are currently focusing on SC isolation from the injury site to investigate their metabolic profile, with the goal of identify possible dysregulated metabolic routes that could modulate macrophage pro-tumoral polarization and function. Ultimately, this work aims to elucidate the intercellular communication networks within the TME driving MPNST initiation and progression, and that could represent novel therapeutic targets.

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Poster 19

Catechol-o-methyltransferase, a potential diagnostic marker and therapeutic target for non-invasive detection of human urothelial carcinoma

Monia Cecati[1], Roberto Campagna[2], Davide Sartini[2], Veronica Pompei[2], Angelo Cormio[2], Giulio Milanese[2], Andrea Benedetto Galosi[2], Monica Emanuelli[2], Valentina Pozzi[2]

[1]Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University, 00166 Rome, Italy; monia.cecati@uniroma5.it; roberto.campagna@uniroma5.it
[2]Department of Clinical Sciences, Polytechnic University of Marche, Ancona, Italy; d.sartini@univpm.it; v.pompei@staff.univpm.it; angelocormio4@gmail.com; g.milanese@univpm.it; a.b.galosi@univpm.it; m.emanuelli@univpm.it; v.pozzi@univpm.it;

Background: Bladder urothelial carcinoma (BUC) is the tenth most frequently diagnosed cancer worldwide, mainly affecting the elderly. This malignancy is characterized by high recurrence and metastatic potential, contributing to poor prognosis. Although cystoscopy is the gold standard for bladder cancer diagnosis and follow-up, it is invasive. Urinary cytology, commonly used alongside cystoscopy, shows low sensitivity, especially for low-grade tumors.

Aim: Consequently, there is a growing need for accurate, non-invasive diagnostic approaches, prompting ongoing efforts to identify and validate novel biomarkers for BUC.

Result: In light of these considerations, we analyzed gene expression profiles in both tumor and non-tumor bladder tissue specimens, as well as urine samples, collected from patients with BUC and healthy controls using microarray analysis. Catechol-O-methyltransferase (COMT) was identified as a significantly upregulated gene in BUC. COMT overexpression was subsequently validated by Real-time PCR and Western blot analyses in both tumor tissues and urine samples from patients with BUC compared with controls. Immunohistochemical analyses are currently underway to further validate these findings. In addition, to investigate the role of this enzyme in the metabolism of BUC cells, the human urothelial carcinoma cell line T24 was transfected with vectors encoding short hairpin RNAs targeting COMT mRNA. The efficiency of COMT knockdown was evaluated by Real-time PCR and the effects of COMT silencing on cell viability were also assessed. Our results showed that BUC samples display a significantly higher COMT expression compared with that detected in controls. Preliminary results suggest that COMT knockdown in BUC cell line is associated to a decrease of cell proliferation.

Conclusions: Data obtained show that the enzyme may represent an interesting tool for BUC, thus highlighting its promising role both as early diagnostic marker and therapeutic target.

Poster 20

Molecular insights into Gremlin-1–driven reprogramming of cancer-associated adipocytes and their interaction with pancreatic ductal adenocarcinoma cells

Martina Codibue[1], Anna Gogna[1], Cosetta Ravelli[1], Michela Corsini[1], Anna Bianchi[2], Jelena Zurkovic[3], Silvia Ministrini[4], Riccardo Nascimbeni[5,4], Roberto Bresciani[1,6], Giorgio Biasiotto[1,6], Stefania Mitola[1] and Elisabetta Grillo[1]

[1]Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
[2]University of Miami Miller School of Medicine, Miami, USA
[3]Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany
[4]General surgery 3, ASST Spedali Civili di Brescia, Brescia, Italy
[5]Department of clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[6]Highly Specialized Laboratory, ASST Spedali Civili di Brescia, Brescia, Italy

Background: The tumor microenvironment is a key driver of cancer progression, including pancreatic ductal adenocarcinoma (PDAC), a highly lethal disease with ~13% 5-year survival rate. Within the TME, the contribution of adipocytes, especially of cancer-associated adipocytes (CAAs), to cancer progression remains unclear for PDAC, although tumor infiltration of adipose tissue is frequent and pathologically assessed for tumor staging. The BMP antagonist Gremlin-1 is overexpressed in PDAC (TCGA PAAD dataset), its expression correlates with mesenchymal markers in patients, and it is enriched in the secretome of aggressive basal-like PDAC cell lines. This suggests not only that Gremlin-1 might be a driver of PDAC, but also that PDAC TME is enriched in gremlin-1 amounts.

Aim: We aim to investigate gremlin-1-driven transformation of adipocytes within the TME of PDAC, and to assess whether this process contributes to tumorigenicity.

Results: Administration of recGrem-1 promotes phenotypic/metabolic changes in *in vitro* adipocytes with a BMP-independent/RTK-dependent mechanism, including increased expression of inflammatory/fibrotic markers (*Tnfa, S100a4, Vim*), eightened oxidative metabolism and reduced lipid content, consistent with a CAA-like phenotype. Similar effects were observed with conditioned medium (CM) from gremlin-1-expressing PDAC cells, together with the upregulation of Hippo pathway genes (*Cyr61, Yap1, Lats2*). In turn, PDAC cells treated with CM from recGrem-1-induced CAAs showed increased proliferative/migratory capacity and oxidative metabolism. Co-culture of PDAC cells with adipocytes in the presence of gremlin-1 led to the upregulation of EMT markers (*Col1a1, Des, Mmp2, Mmp14*). In PDAC patients (TCGA PAAD dataset), a previously identified CAA gene signature correlates with GREM1 expression.

Conclusions: Our findings indicate a novel role for Gremlin-1 in the induction of CAAs in PDAC, further supporting tumorigenicity.

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Poster 21

Unraveling TET-driven epigenetic rewiring in cancer adaptation upon loss of respiratory complex I

Simona Corrà[1,2], Anna Carolina Emi de Lima Tanada[3], Federico Manai[4], Davide Arfini[1,2], Beatrice Cavina[1,2], Francesca Nanetti[1,2], Anna Maria Porcelli[5], Giuseppe Gasparre[1,2], Enrica Calura[4], Rossella Tricarico[3,6], Ivana Kurelac[1,2]

[1]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Via Massarenti 9, Bologna, Italy.
[2]Center for Applied Biomedical Research (CRBA), University of Bologna, Bologna, Italy.
[3]Department of Biology, University of Padova, 35121 Padua, Italy.
[4]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, 27100 Pavia, Italy.
[5]Department of Pharmacy and Biotechnology (FABIT), University of Bologna, Bologna, Italy.
[6]Laboratory of Epigenetics, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy.

Background: Targeting respiratory complex I (CI) is a promising anticancer strategy in solid tumors, but with time triggers compensatory responses that allow cancer cell survival. Since loss of CI increases levels of α-ketoglutarate and oxygen, both involved in regulating Ten-Eleven Translocation (TET) DNA demethylases, we here hypothesized TET-dependent active DNA demethylation may be driving survival responses triggered upon CI inhibition.

Aim: Test whether CI loss enhances TET activity, promoting a pro-survival transcriptional program, and demonstrate TET inhibition improves the efficacy of CI targeting.

Results: To assess TET role under CI deficiency, CI knockout (KO) of ovarian cancer model was generated and grown with respective control *in vivo*. Histological staining and methylome analysis showed, respectively, higher DNA demethylation and broad hypomethylation in CI KO xenografts, suggesting enhanced TET activity. Importantly, CI restoration reversed this phenotype, supporting a causal link between CI loss and epigenetic rewiring. Based on this phenotype, TET2 was selected as an initial functional candidate. *TET2* knockdown (KD) caused mild proliferation decrease in CI KOs, both by viability and spheroids formation *in vitro* assays, indicating a partial TET2-mediated contribution to cancer proliferation in our conditions. Multi-omic analysis identified 55 demethylated and upregulated genes in CI KO xenografts (>60% with known pro-survival functions), whose *in vitro* validation in CI KO and *TET2* KD models identified three genes putatively regulated by TET2.

Conclusions: CI KO induces TET-mediated active DNA demethylation of various prosurvival genes, revealing a possible TET-mediated compensatory role in CI-deficient cancers. However, the mild effect of TET2 KD suggests redundancy or cooperation with other TET enzymes, supporting broader investigation of TET-dependent demethylation as a targetable adaptive pathway.

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Poster 22

A dual-action CO-releasing APR-246 conjugate targets mitochondria and triggers multimodal programmed cell death in PDAC

Federica Danzi[1], Adriana Celesia[1], Filippo Piccoli[1], Raffaella Pacchiana[1], Chiara Tesoriero[2], Gerlinde Grabmann[3], Antonino Aparo[4], Maria Teresa Scupoli[4], Andrea Vettori[2], Thomas Köcher[3], Chiara Riganti[5], Konstantin Chegaev[6], Alessandra Fiore[1*] and Massimo Donadelli[1*]

[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy;
[2]Department of Biotechnology, University of Verona, Verona, Italy;
[3]Vienna BioCenter Core Facilities GmbH, Vienna, Austria;
[4]Research Center LURM (Interdepartmental Laboratory of Medical Research), University of Verona, Verona, Italy
[5]Department of Oncology, University of Torino, Torino, Italy;
[6]Department of Drug Science and Technology, University of Torino, Torino, Italy.

* These authors contributed equally
Presenting author: Federica Danzi, federica.danzi@univr.it

Background: Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest cancers, with *TP53* mutations occurring in 50–70% of cases and contributing to tumor progression, metabolic rewiring, and therapy resistance. Current treatments still provide limited clinical benefits, highlighting the urgent need for innovative therapeutic strategies.

Aim: To address this challenge, we developed a novel carbon monoxide-releasing molecule (CORM) conjugated with APR-246, a mutant p53 reactivator currently in clinical trials, designed to enhance anticancer activity through dual targeting of mutant p53 and cellular metabolism.

Results: We first demonstrated that the conjugate, APR-246-CORM, exhibits higher cytotoxicity than APR-246 or the CORM alone across several *TP53*-mutant PDAC cell lines, while showing reduced toxicity in non-tumoral cells, suggesting tumor selectivity. Importantly, CORM uptake and intracellular CO accumulation were confirmed by flow cytometry and confocal microscopy. APR-246-CORM markedly increased cellular and mitochondrial ROS levels and induced mitochondrial membrane hyperpolarization, consistent with profound mitochondrial dysfunction. In parallel, Seahorse ATP rate assays demonstrated a metabolic shift toward glycolysis over oxidative phosphorylation. Furthermore, RNA-seq analysis and untargeted metabolomics revealed a distinct transcriptional and metabolic signature in APR-246-CORM-treated cells, enriched for autophagy, apoptosis, ferroptosis and p53 signaling pathways. Finally, preliminary zebrafish studies showed no overt toxicity at high doses while confirming *in vivo* uptake of the CORM moiety.

Conclusions: Overall, APR-246-CORM enhances APR-246 antitumor activity by promoting mitochondrial dysfunction and multimodal programmed cell death, supporting its potential at a novel therapeutic strategy for *TP53*-mutant PDAC.

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Poster 23

Title The F_1F_o-ATPase inhibitor protein, IF_1, plays no role in the metabolic reprogramming of cancer cells, but is essential for their adaptation to low oxygen tension

Valentina Del Dotto, Silvia Grillini, Riccardo Righetti, Martina Grandi, Valentina Giorgio, Giancarlo Solaini and Alessandra Baracca

Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, I-40126, Italy
E-mail: valentina.deldotto2@unibo.it

Background: Tumor cells are characterized by uncontrolled growth, resistance to apoptosis and increased motility, features closely associated with metabolic adaptations, making metabolic reprogramming a hallmark of cancer. IF1, the endogenous inhibitor of mitochondrial ATP synthase, is frequently overexpressed in cancers and has been proposed to exert a pro-oncogenic role through different mechanisms.

Aim: Recently, we have investigated in depth the possibility that IF1 can express its pro-oncogenic action as inhibitor of the ATP synthesis activity in normoxia, contributing to the reprogramming of energy metabolism (Warburg effect), and the biochemical mechanisms underlying its role under hypoxic/anoxic conditions, that has been previously shown.

Results: We prepared stably IF1-silenced clones and compared their metabolic features with those of three different cancer cell lines: 143B, HCT116 and HeLa. No significant difference was observed in the glycolytic flux between IF1-silenced and control cells under both basal condition and oligomycin inhibited OXPHOS, as shown by the measurements of both glucose consumed and lactate released. In addition, we observed no changes in functional parameters, such as respiration (OCR) and ATP synthesis rate, and in steady-state cellular ATP levels, even after oxidative phosphorylation or glycolysis inhibition. On the contrary, under anoxia mimicking conditions, only in IF1-expressing cells the energy charge is sufficiently preserved to promote survival and allow the renewal of the mitochondrial mass. Notably, the presence of IF1 conferred a proliferative advantage to anoxic cells highly dependent on oxidative phosphorylation when reoxygenation occurs

Conclusions: Our findings exclude that IF1 inhibits the ATP synthase when the enzyme is working physiologically (ATP synthesis), but, allowing energy preservation and promoting mitochondrial renewal in hypoxic/anoxic cells, can favor proliferation and tumor growth.

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Poster 24

The Sweet side of epigenetics: BET Proteins as Regulators of N-Glycosylation

Angela De Cristofaro[1], Emanuele Salvi[1], Maria Rosa Ciriolo[1], and **Enrico Desideri**[2]

[1]Department of Biology, University of Rome Tor Vergata angeladecr@gmail.com (ADC); emanuele.salvi.93@gmail.com (ES); ciriolo@bio.uniroma2.it (MRC)
[2]Department of Life Sciences, Health and Health Professions, Link Campus University; e.desideri@unilink.it (ED)

Background: Bromodomain and extra-terminal (BET) proteins are epigenetic readers that recognize acetylated lysine residues on histone tails and recruit protein complexes to modulate gene expression. Since the discovery that the MYC proto-oncogene is a downstream target, BET inhibition has been extensively explored as an anticancer strategy. However, clinical application remains hindered by limited monotherapy efficacy and acquired resistance.

Aim: We analyzed multiple RNA-seq datasets from diverse cancer cell lines treated with various BET inhibitors to identify consistently deregulated pathways and reveal novel molecular mechanisms of resistance or acquired vulnerabilities.

Results: RNA-seq analysis revealed that genes associated with the ER, Golgi , and lysosomes are the most significantly enriched among those upregulated following BET inhibition. These organelles are interconnected because the synthesis, modification and sorting of lysosomal proteins take place in the ER and Golgi. Analysis of Lysosome-Associated Membrane Proteins (LAMPs) showed that BET inhibition induced an increase in LAMP2 expression and a significant shift in the molecular weight of both LAMP1 and LAMP2. This shift is driven by enhanced N-glycosylation, specifically an increase in complex N-glycans. This phenomenon extends beyond the lysosome, also affecting plasma membrane proteins. Mechanistically, BET inhibition transcriptionally upregulates Golgi mannosidases, particularly MAN1A1 and MAN1C1, which are essential for the maturation of complex N-glycans. Functionally, this altered glycosylation causes lysosomal fragility, sensitizing cells to rupture in response to lysosomotropic agents.

Conclusions: Our findings reveal a novel role for BET proteins in regulating protein glycosylation. Given that N-glycosylation is a fundamental post-translational modification, this BET-dependent glycan remodeling can significantly impact cellular homeostasis and reveal potential therapeutic opportunities.

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Poster 25

Beyond inhibition: targeted protein degradation to reshape the tumor microenvironment

Valeria Di Giacomo[1], Laura Marrone[1], Simona Caggiano[1], Felix Hausch[2], Marialuisa Alessandra Vecchione[1], Rosetta Abate[1], Federica Aracri[1], Maria Fiammetta Romano[1] and Simona Romano[1]

[1]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II – Italy
[2]Technical University Darmstadt Institute of Organic Chemistry and Biochemistry - Germany

Background: Tumour-associated macrophages (TAMs) are central regulators of immune suppression in the tumour microenvironment (TME). M2-like TAMs promote tumour progression by suppressing cytotoxic T-cell responses and facilitating immune evasion. We identified the alternatively spliced FKBP5 isoform FKBP51s as a regulator of macrophage polarization, selectively upregulated in M2 macrophages and linked to immunosuppressive signalling.

Aim: To evaluate the effects of FKBP51s targeting through in vivo FK1-domain inhibition with SAFit compounds and in vitro isoform-selective degradation using PROTACs, with a focus on TAM reprogramming.

Results: Dual in vivo inhibition of FKBP51 isoforms enhanced immunosuppressive features of the TME, including increased M2-like macrophage accumulation, reduced perforin expression, and CD8+ T-cell exhaustion. In contrast, selective FKBP51s degradation by five PROTACs reprogrammed M2 macrophages toward an M1-like phenotype, accompanied by increased STAT1 activation, reduced STAT3/STAT6 signalling, and enhanced pro-inflammatory mediator production.

Conclusions: FKBP51s emerges as a novel regulator of macrophage-driven immune suppression in the TME. Selective PROTAC-mediated degradation of FKBP51s restores pro-inflammatory macrophage activity and may represent a promising strategy to therapeutically reprogram the immunosuppressive tumour microenvironment.

Poster 26

NGFR-driven lipid metabolic reprogramming in CR-CSCs: implications for chemoresistance in colon cancer

Noemi Di Miceli[1], Ornella Roberta Brancato[1], Francesco Verona[1], Kimiya Shams[1], Giorgio Stassi[1] and Simone Di Franco[1]

[1]Department of Precision Medicine in Medical, Surgery and Critical Area (Me.Pre.C.C.), University of Palermo, Italy.

Introduction: The nerve growth factor receptor (NGFR) plays a key role in neuronal plasticity and is implicated in tumor progression in colon cancer. Within the tumor bulk, colorectal cancer stem cells (CR-CSCs) are characterized by self-renewal capacity, tumor growth promotion, metastasis, and chemoresistance. Recent studies have highlighted that CR-CSCs reprogram their lipid metabolism to sustain stemness, enhance metastasis, and confer chemotherapy resistance. Notably, CR-CSCs accumulate lipid droplets (LDs) more than healthy cells. Under metabolic stress, LDs release free fatty acids, which can be utilized for ATP generation via fatty acid oxidation (FAO). Dysregulated lipid metabolism is a critical factor in cancer development, and increased lipid uptake enhances the tumor-initiating capacity of CR-CSCs.

Aim: Investigate NGFR in colorectal cancer (CRC), focusing on its role in lipid metabolism and its contribution to chemoresistance in colorectal cancer stem cells (CR-CSCs).

Results and Discussion: Analysis of public databases (GEPIA) identified genes associated with NGFR expression in CRC, including lipid metabolism regulators such as CD36, CIDEA, and FABP4. GSEA revealed enrichment in adipogenesis, lipid localization, and transport pathways. Transcriptomic analysis of NGFR-overexpressing CR-CSCs confirmed upregulation of lipid-related genes (FASN, FADS1, FADS2, PLIN1, SCD1), indicating NGFR involvement in lipid metabolism. Nanolive imaging and Nile Red staining showed increased lipid droplet accumulation, while FAO Blue staining indicated enhanced β-oxidation. Furthermore, NGFR overexpression promoted chemoresistance to standard treatments, suggesting a key role of lipid metabolism in therapy resistance.

Conclusions: NGFR boosts lipid metabolism in CR-CSCs, suggesting its critical role in regulating lipid metabolism, which may contribute to chemoresistance in CRC. NGFR could represent a potential therapeutic target in overcoming chemoresistance.

Poster 27

Targeted inhibition of mitochondrial Complex I impairs tumor growth with low cytotoxicity

Giorgia Esposito[1], Beatrice Cavina[1], Manuela Sollazzo[2], Simona Corrà[1], Laura Lanteri[2], Francesca Nanetti[1], Monica De Luise[1], Maria Iorio[1], Gaia Tioli[2], Hamid Razi Nasiri[3], Anna Ghelli[2], Luisa Iommarini[2], Anna Maria Porcelli[2], Ivana Kurelac[1] and Giuseppe Gasparre[1]

[1]University of Bologna, DIMEC, Bologna, Italy; ivana.kurelac@unibo.it; giuseppe.gasparre3@unibo.it
[2]University of Bologna, FABIT, Bologna, Italy; annamaria.porcelli@unibo.it
[3]Goethe University, Frankfurt, Germany; nasiri@nmr.uni-frankfurt.de

Background: Inhibition of mitochondrial respiratory complex I (CI) is an emerging anticancer strategy supported by preclinical and epidemiological evidence. However, no CI inhibitors are currently used in clinical practice, and key issues such as specificity and optimal dosing, without collateral effects, remain unresolved.

Aim: In this study, we present the antiproliferative activity and mechanism of action of two novel and specific CI inhibitors, EVP-4593 (EVP) and QA2, with the aim to obtaining effective and potent anticancer candidates, with a reduced systemic toxicity.

Results: QA2 and EVP demonstrated antitumor activity across five solid tumor models, abolishing mitochondrial respiration and inhibiting cell growth. EVP emerged as the most potent compound compared with QA2 and IACS-010759. However, at high concentrations (≥ 20 μM), EVP induced off-target cytotoxic effects, whereas QA2 maintained a cytostatic profile, without ROS production or apoptosis. Moreover, QA2 displayed lower toxicity than IACS-010759, whose clinical development was discontinued due to neurotoxic side effects. When treatment effects were associated with Complex I inhibition, the antiproliferative activity of both molecules depended on CI blockade and did not induce toxic effects in non-neoplastic cells. Consistent results were obtained ex vivo using the U-Cup system on patient-derived ovarian cancer samples, showing reduced proliferation. In vivo, QA2 and EVP reduced tumor growth and improved survival in the HCT xenograft model. Pharmacokinetic and pharmacodynamic studies in the OV90 xenograft model are ongoing to optimize dosage regimens, define the therapeutic potential of these compounds in combinatorial anticancer strategies, and support their safe translation into human clinical applications.

Conclusion: Overall, these findings support QA2 and EVP as promising therapeutic strategies for chemotherapy-resistant patients, particularly as second-line treatments or maintenance therapies.

Poster 28

Development and Evaluation of Albumin Nanoparticles Encapsulating Fenretinide for Enhanced Efficacy and Combination Therapy in Glioblastoma

Francesca Farioli[1], Lorenzo Anconelli[1], Martina Rossi[1], Giovanna Farruggia[1], Emil Malucelli[1], Concettina Cappadone[1], Paolo Blasi[1], Cecilia Garofalo[2], Stefano Iotti[1] and Isabella Orienti[1]

1Department of Pharmacy and Biotechnology, University of Bologna, Bologna Italy 2IRCCS Institute of Neurological Sciences of Bologna, Bologna, Italy

Background: Glioblastoma (GBM) is one of the most aggressive and lethal malignancies, and its treatment is severely hindered by the poor therapeutic efficacy and frequent resistance to standard temozolomide (TMZ) regimens. Fenretinide (Fen) exhibits promising antitumor properties, but its clinical utility is hindered by poor aqueous solubility, which restricts its bioavailability. Nanotechnology offers a powerful strategy to overcome these pharmacokinetic barriers. Furthermore, combining nanotechnology-based therapies with conventional chemotherapy represents a crucial approach to circumvent GBM heterogeneity and enhance treatment outcomes.

Aim: Develop human serum albumin nanoparticles to encapsulate Fen (HSA-F), to improve its bioavailability and antitumor efficacy and evaluate the therapeutic efficacy of HSA-F on 3D glioblastoma models, in combination with frontline agents, including TMZ, bortezomib and regorafenib.

Results: HSA-F markedly enhanced Fen solubility and was characterized by nanoscale dimensions in the range of 100-200 nm, suitable for accumulation in solid tumors by the enhanced permeability of angiogenic capillaries. In 3D models HSAF demonstrated significantly enhanced antitumor activity and superior cytotoxicity compared to free Fen in disrupting spheroid survival. Among the tested drug combinations, the co-administration of HSA-F with the proteasome inhibitor bortezomib emerged as the most promising regimen, yielding a powerful synergistic effect that drastically reduced spheroid viability.

Conclusions: HSA-F nanoparticles improved Fen solubility and bioavailability as demonstrated by the higher antitumor activity in 3 D glioblastoma models. In combination with bortezomib, HSA-F exhibited a powerful synergistic effect, which drastically reduced spheroid viability. Moreover, the dimensions of HSA-F nanoparticles are suitable for accumulation in brain tumors where the brain-barrier is frequently permeabilized by the remodelling tumor processes.

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Poster 29

Bi-directional regulation between NAD/NAMPT and IFN-γ/PD-L1 axes via BRD4/IRF1 and mitochondrial respiration in metastatic cutaneous melanoma

Irene Fiorilla[1], Beatrice Ghezzi[1], Nicoletta Tommasi[1], Enrico Moiso[2], Lidia Avalle[1], Giovanna Carrà[3], Filippo Ugolini[4], Edoardo Calussi[4], Alessia Ponzano[1], Alberto Maria Todesco[1], Filippo Casone[1], Luca Ponzone[5], Sabrina Digiovanni[6], Anna Szumera-Ciećkiewicz[7], Maria Cavaletto[8], Paolo Ettore Porporato[5], Chiara Riganti[6], Daniela Massi[9], Enzo Calautti[5] and Valentina Audrito[1,10]

[1]Department of Science and Technological Innovation, University of Eastern Piedmont, Alessandria, Italy.
[2]Memorial Sloan Kettering Cancer Center, New York, NY, USA.
[3]San Luigi Gonzaga Hospital, Regione Gonzole, Orbassano, Italy. Department of Clinical and Biological Sciences, University of Turin, Italy.
[4]Histopathology and Molecular Diagnostics, Careggi University Hospital, Florence, Italy.
[5]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center "Guido Tarone", University of Turin, Torino, Italy.
[6]Department of Oncology, Molecular Biotechnology Center "Guido Tarone", University of Turin, Torino, Italy.
[7]Department of Pathology and Laboratory Medicine, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland.
[8]Department of Sustainable Development and Ecological Transition, University of Eastern Piedmont, Vercelli, Italy.
[9]Section of Pathology, Department of Health Sciences, University of Florence, Florence, Italy.
[10]Research Laboratories, Research and Innovation Department (DAIRI), Public Hospital Azienda Ospedaliero Universitaria "SS. Antonio e Biagio e Cesare Arrigo", Alessandria, Italy.

Background and Aims: Metastatic cutaneous melanoma (MCM) is primarily treated with BRAF/MEK inhibitors and immune checkpoint inhibitors (ICIs), but the long-term efficacy of these therapies is often limited by acquired resistance. Nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in NAD biosynthesis, is frequently upregulated in MCM, supporting metabolic rewiring and targeted therapy resistance. Emerging evidence links the NAD/NAMPT axis to interferon-γ (IFN-γ) signaling, a pathway with dual and context-dependent effects in cancer, ranging from promoting cytotoxic immune activity to inducing PD-L1–mediated immune evasion. However, the functional interplay between NAD/NAMPT axis and IFN-γ signaling in MCM cells is still poorly understood.

Methods: Transcriptomic, bioinformatic, biochemical, molecular, and immunohistochemical approaches were integrated to dissect the bi-directional regulation between NAMPT and IFN-γ. These analyses combined IFN-γ–treated melanoma cell lines, TCGA datasets, and a tissue microarray (TMA) comprising over 100 metastatic melanoma samples. The roles of BRD4 and IRF1 were validated through pharmacological and genetic inhibition as well as chromatin immunoprecipitation (ChIP) assays. Metabolic analyses were performed to assess the NAD^+/NAMPT-dependent regulation of IFN-γ–induced mitochondrial respiration.

Results: IFN-γ treatment induced a transcriptional enrichment of the nicotinate/nicotinamide metabolism pathway and increased both intracellular and extracellular NAMPT (iNAMPT/eNAMPT) levels, together with PD-L1 and CXCL10 expression. TCGA analysis revealed a strong correlation between NAMPT and IFN-γ–responsive genes (STAT1, IRF1, CD274/PD-L1), confirmed at the protein level in the TMA cohort. Mechanistically, IFN-γ promoted BRD4/IRF1 recruitment to the NAMPT promoter, while BRD4 inhibition/silencing suppressed both basal and inducible NAMPT expression. Conversely, NAMPT inhibition impaired STAT1 activation and IFN-γ–responsive gene transcription. Functionally, IFN-γ enhanced mitochondrial complex I activity and oxygen consumption, which were both reduced upon NAMPT blockade.

Conclusions: Collectively, our findings reveal a previously unrecognized feed-forward loop linking NAMPT/NAD axis and BRD4/IRF1 in IFN-γ signaling regulation. Such metabolic/immune crosstalk may open new venues for overcoming therapeutic resistances in MCM.

Poster 30

From Metabolic Heterogeneity to Targeted Treatment: Insights from NSCLC and Glioblastoma Models

Luca Frattaruolo[1], Graziantonio Lauria[1], Alessandra Ferramosca[2], Paola Lunetti[2], Graziana Assalve[2], Marco Fiorillo[1], Vincenza Dolce[1], Vincenzo Zara[2] and Anna Rita Cappello[1]

[1]Department of Pharmacy, Health & Nutritional Sciences, University of Calabria, 87036 Rende (CS), Italy; luca.frattaruolo@unical.it
[2]Department of Experimental Medicine, University of Salento, 73100 Lecce (LE), Italy.

Background: Since cancer cells undergo extensive metabolic rewiring to sustain proliferation, evade cell death, and adapt to the tumor microenvironment, understanding these alterations is essential to elucidate the mechanisms driving tumor initiation and progression and to identify potential therapeutic targets; consequently, cancer metabolism is increasingly recognized as a pivotal component of tumor biology.

Aim: The aim of this work was to characterize the metabolic profiles of non-small cell lung cancer (NSCLC) and glioblastoma (GBM), two highly aggressive tumors with marked intra- and inter-tumoral metabolic heterogeneity[1,2], and to explore the relationship between metabolic phenotypes and drug response.

Results: Metabolic phenotypes of several NSCLC and GBM cell lines were assessed through integrated molecular and functional analyses, which highlighted marked heterogeneity across the different models, with distinct patterns of metabolic pathway utilization supporting cancer cell proliferation. Notably, these metabolic differences were associated with variable sensitivity to thioalbamide, a specialized microbial peptide that acts as inhibitor of oxidative phosphorylation, suggesting a link between metabolic state and treatment response.

Conclusions: These findings highlight the importance of integrating metabolic profiling into cancer research. A deeper understanding of metabolic reprogramming and tumor heterogeneity may support the development of more effective, personalized therapeutic strategies.

References

1. Cai, H., Zhang, F., Xu, F., & Yang, C. (2025). *Frontiers in Oncology*, *15*, 1564226.
2. D'Aprile, S., Denaro, S., Gervasi, A., Vicario, N., & Parenti, R. (2025). *Frontiers in Cell and Developmental Biology*, 13, 1535073.

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Poster 31

NMR-based metabolomics reveals that TG2 inhibition triggers cell line-specific metabolic reprogramming in Triple-Negative Breast Cancer

Mariana Gallo[1], Elena Ferrari[1], Cristina Zuccato[2], Jeffrey W. Keillor[3], Thelma A. Pertinhez[1] and Nicoletta Bianchi[2]

[1]Department of Medicine and Surgery, University of Parma, Italy
[2]Department of Translational Medicine, University of Ferrara, Italy
[3]Department of Chemistry and Biomolecular Sciences, University of Ottawa, Canada

Background: Transglutaminase 2 (TG2) is a multifunctional enzyme involved in cellular and metabolic processes. In cancer, TG2 promotes epithelial-mesenchymal transition, invasiveness, adhesion, and metastatic competence. In breast cancer (BC), TG2 is frequently overexpressed and associated with metastasis, therapy resistance, and poor prognosis, making it a promising therapeutic target.

Aim: This study investigates the metabolic effects of TG2 inhibition by AA9, a novel inhibitor, in two aggressive triple-negative breast cancer (TNBC) cell lines, MDA-MB-231 and MDA-MB-436. By integrating NMR-based metabolomic profiling of both intracellular and extracellular compartments, we provide a comprehensive characterization of the metabolic alterations induced by TG2 inhibition.

Results: AA9 induced cell-specific metabolic responses in both TNBC cell models. In MDA-MB-231, TG2 inhibition reduced glucose consumption, lactate production, and TCA intermediates, suggesting compensatory anaplerotic adaptations. Intracellular branched-chain amino acids (BCAAs) were accumulated alongside reduced glutamate and increased secretion of BCAA catabolites. In MDA-MB-436, glucose consumption was unchanged, while lactate increased and TCA intermediates decreased, indicating enhanced aerobic glycolysis. Myo-inositol and taurine increased, suggesting osmotic adaptation. In both cell lines, GSSG decreased, consistent with impaired GPX4 activity, whereas NAD^+ increased. Changes in glycine and glutamine further suggest redox stress and amino acid rewiring. AA9 also altered lipid metabolism: MDA-MB-231 showed changes consistent with phospholipid turnover and membrane stress, whereas MDA-MB-436 displayed a distinct lipid adaptation pattern. **Conclusions:** TG2 inhibition induces cell-specific metabolic rewiring in aggressive TNBC cells, revealing distinct metabolic vulnerabilities and supporting TG2 as a promising therapeutic target.

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Poster 32

Macrophages cell lysis: a step towards lysosomes extraction to understand multiple myeloma aggressiveness

Laure Gauthier-Manuel[1], Nour Kassab[1], Batuhan Calik[1], Cristina Visentin[1], and Stefano Ricagno[1]

Affiliation 1: Università degli Studi di Milano laure.gauthier-manuel@unimi.it, nouralhoda.kassab@studenti.unimi.it, batuhan.calik@studenti.unimi.it, cristina.visentin@unimi.it, stefano.ricagno@unimi.it

Multiple myeloma (MM) is a blood cancer affecting plasma cells which evolves toward general organ damage resulting in patient's poor prognosis. A notable MM symptom is a circulating concentration of Beta-2-microglobulin (β2m) over 3 µg/ml. Tumor-associated macrophages (TAMs) internalize this protein for intracellular degradation after phagosome–lysosome fusion. However, the associated acidic and crowded environment enhances the amyloidogenic properties of β2m, leading to lysosomal membrane rupture and triggering cellular stress and inflammatory response in MM. Therefore, studying the morphology and biochemical composition of the lysosomal lumen in TAM is crucial to understanding the amyloidogenic transition of β2m, a process implicated in MM progression. For this purpose, lysosomes must be isolated from differentiated macrophage cells while preserving their integrity following cell lysis. Mechanical lysis with glass homogenizer, sonication and plasma membrane solubilization were the three methods screened to optimize a smooth cell lysis. Even with a high number of strokes, mechanical lysis was inefficient to rupture cells. Sonication showed a good reproducibility in cell breakage, but lysosomal loss was reported. Instead, membrane solubilization is less reliable but most of the lysosomes extracted were present in the final fraction. In conclusion, we established two cell lysis procedures for lysosomes extraction from macrophages prior to their purification and subsequent biophysical analyses.

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Poster 33

TRPM7 Channel Activity Drives Metabolic Rewiring and ROS Production in Breast Cancer Cell Lines

Marialaura Giannaccari[1], Chiara Florindi[2], Francesco Lodola[2], Francesco Moccia[3], Livia Visai[1,4] and Nora Bloise[1,4]

[1]Molecular Medicine Department (DMM), Centre for Health Technologies (CHT), Unità di Ricerca (UdR) INSTM, Operative Unit (OU) of the Interuniversity Center for the Promotion of the 3Rs Principles in Teaching and Research (Centro 3R), University of Pavia, 27100 Pavia, Italy;
marialaura.giannaccari01@universitadipavia.it; nora.bloise@unipv.it; livia.visai@unipv.it
[2]Department of Biotechnology and Bioscience, University of Milano-Bicocca, 20126 Milan, Italy;
c.florindi@campus.unimib.it; francesco.lodola@unimib.it
[3]Dipartimento di Medicina e Scienze della Salute "V. Tiberio", Università degli Studi del Molise, 86100 Campobasso; francesco.moccia@unimol.it
[4]UOR6 Nanotechnology Lab., Department of Prevention and Rehabilitation in Occupational Medicine and Specialty Medicine, Istituti Clinici Scientifici Maugeri IRCCS,27100 Pavia, Italy nora.bloise@unipv.it; livia.visai@unipv.it

Background: The bifunctional chanzyme Transient Receptor Potential Melastatin 7 (TRPM7) is a pivotal regulator of calcium homeostasis, recently implied in driving oncogenesis. Up-regulated TRPM7 expression strongly correlates with unfavorable clinical outcomes in breast cancer patients [doi:10.3390/cancers13246322], indicating a crucial role in malignancies.

Aim: This study investigates the role of the TRPM7 channel in breast cancer progression, with a particular focus on its contribution to ROS production and metabolic alterations.

Results: TRPM7 transcript levels were analyzed via qPCR in breast cancer cell lines (MCF-7, MDA-MB-231, SK-BR-3) and the non-tumorigenic control line MCF-10A. Among the tested models, MCF-7 cells exhibited a markedly elevated upregulation of TRPM7. Intracellular calcium flux was monitored using Fura-2 fluorometry following pharmacological modulation. The data showed significant fluctuations in Ca^{2+} dynamics upon precise chemical activation by Naltriben or NS8593-mediated blockade. Small interfering RNA (siRNA) was employed to silence TRPM7, after which cell viability was detected by CCK-8 assays and downstream transcriptional changes were evaluated by qPCR. Genetic silencing of TRPM7 substantially suppressed breast cancer cell viability, channel responsiveness, and altered related gene expression. Finally, metabolic impacts were investigated through ROS quantification and targeted metabolite profiling via colorimetric and mass spectrometry platforms. Flow cytometry and confocal imaging revealed increased ROS production in silenced cells, while GC-MS analysis showed altered metabolic pathways, including glycolysis, the TCA cycle, and fatty acid metabolism.

Conclusions: Our findings revealed a connection between TRPM7 channel activity and tumor metabolic progression, highlighting this chanzyme as a promising therapeutic target in breast oncology.

Poster 34

TARGETING METABOLIC DYSREGULATION TO BYPASS IMMUNE ESCAPE IN HUMAN COLORECTAL CARCINOMA

Grillo C.[1], Iozzo M.[1], Ippolito L.[1], Pranzini E.[1], Gangarossa G.[1], Bertoli G.[1], Venditti G.[1], Roccabianca S.[1], Comito G.[1], Esposito F.[3], Landriscina M.[2], Chiarugi P.[1], Giannoni E.[1]

[1]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy. caterina.grillo@edu.unifi.it
[2]Unit of Medical Oncology and Biomolecular Therapy, Department of Medical and Surgical Sciences - Policlinico Riuniti, University of Foggia, Italy.
[3]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples 80131

Background: Colorectal cancer (CRC) is a heterogeneous gastrointestinal malignancy classified into four consensus molecular subtypes (CMS). CMS1 subtype is characterized by high microsatellite instability, immune activation and sensitivity to immune checkpoint inhibitors (ICIs). Conversely, CMS3 subtype exhibits metabolic dysregulation and resistance to ICIs. The ability of cancer cells to undergo metabolic alterations contributes to reshaping the tumor microenvironment, promoting cell proliferation, metastasis and immune escape.

Aim: Our aim is to demonstrate how metabolic rewiring, in particular lipid metabolism (LM), contributes to immunoescaping in CRC CMS3 subtype and to identify lipid metabolites involved in immunesuppression.

Results: RNA-seq analysis identified an enrichment in LM-related genes in CMS3 cell lines compared to CMS1, including those encoding Fatty Acid Binding Protein 1 (FABP1), Perilipin 2, Monoacylglycerol Lipase (MAGL) and Fatty Acid Amide Hydrolase (FAAH). Consistently, BODIPY staining showed a marked lipid droplets (LDs) accumulation in CRC CMS3. However, no significant changes were observed in fatty acid (FA) anabolic enzymes, suggesting that LD accumulation mainly depends on increased extracellular lipid uptake, potentially mediated by FABP1. This lipid-enriched phenotype was associated with immunosuppressive behavior, as CD8+ T cells were reduced when co-cultured with CMS3 versus CMS1 cell lines. To investigate whether lipid mediators contributed to this effect, targeted lipidomics analysis revealed elevated endocannabinoids, arachidonic acid (AA), and prostaglandin E2 (PGE2) in CMS3 cells, supporting activation of a lipid mediator pathway. Consistently, ELISA analysis of CRC conditioned media confirmed increased PGE2 levels in CMS3 cultures, suggesting that FABP1-mediated FA uptake may promote MAGL/FAAH-dependent AA–PGE2 production, contributing to immune evasion.

Conclusions: We identify LM as a potential driver of immune escape in CMS3 CRC, likely through activation of the AA/PGE2 axis, indicating a target to restore antitumor immune responses.

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Poster 35

Muscle iron export as a gatekeeper of cancer metastasis

Alfonso Scalera[1], **Sijia Guo**[1], Myriam Hsu[1], Giovanna Carrà[2], Emanuela Tolosano[1], Alessio Menga[3] and Paolo Ettore Porporato[1]

[1]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center "Guido Tarone", University of Torino, 10126 Torino, Italy; paolo.porporato@unito.it
[2]San Luigi Gonzaga Hospital, 10043 Orbassano, Italy; giovanna.carra@unito.it
[3]Department of Health Sciences, Università del Piemonte Orientale UPO, 28100 Novara, Italy; alessio.menga@uniupo.it

Background: Metastasis accounts for over 90% of cancer mortality, yet how peripheral metabolic organs remotely govern metastatic dissemination remains poorly understood. In cancer-bearing hosts, skeletal muscle dysfunction perturbs systemic iron homeostasis and is accompanied by iron-depleted dysfunctional mitochondria and induction of iron exporters such as FLVCR1a, raising the possibility that muscle heme metabolism contributes to metastatic competence.

Aim: To determine whether skeletal muscle heme metabolism regulates metastatic spread and to define the systemic mechanisms linking muscle iron handling to cancer progression.

Results: In Lewis lung carcinoma (LLC)-bearing mice, muscle-specific deletion of FLVCR1a (mKO) did not alter primary tumor growth but markedly reduced pulmonary metastasis. This phenotype was associated with enhanced vascular normalization, consistent with impaired tumor cell intravasation. Mechanistically, FLVCR1a ablation suppressed muscle-derived erythroferrone (ERFE), leading to increased hepatic hepcidin expression, restricted bone marrow iron availability, and defective granulopoiesis. As a consequence, neutrophil abundance and effector function were profoundly compromised. Remarkably, intravenous administration of mini-hepcidin in wild-type LLC tumor-bearing mice phenocopied the anti-metastatic effects observed in mKO animals, establishing a central role for the ERFE-hepcidin-iron axis in metastatic seeding.

Conclusions: These findings identify skeletal muscle as a systemic regulator of cancer metastasis. Through an FLVCR1a–ERFE–hepcidin signaling axis, muscle heme metabolism controls iron bioavailability and innate immune competence in a non-cell-autonomous manner, uncovering muscle–iron crosstalk as a therapeutic vulnerability in aggressive cancer.

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Poster 36

Primary tumor lactic acid selects dormant prostate cancer cells

Luigi Ippolito[1], Erica Pranzini[1], Giulia Gangarossa[1], Marta Iozzo[1], Giuseppina Comito[1], Giacomo Venditti[1], Sara Roccabianca[1], Elisa Pardella[1], Matteo Lulli[1], Alice Santi[1], Elisa Giannoni[1], and Paola Chiarugi[1]

[1]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Viale Morgagni 50, 50134, Florence, Italy luigi.ippolito@unifi.it

Background Dormant disseminated cancer cells (DCCs) drive metastatic relapse and therapy resistance in prostate cancer (PCa). Lactic acid (LA), enriched in primary tumors (PTs), promotes invasive and stem-like traits through metabolic and epigenetic reprogramming, but its role in dormancy is unclear.

Aim To determine whether LA regulates PCa dormancy and how LA availability in primary and metastatic sites affects dormancy and metastatic awakening.

Results LA induced a reversible quiescent state in PCa cells, characterized by reduced proliferation and increased dormant markers and therapy tolerance. Mechanistically, LA upregulated the dormancy regulator COUP-TF1. COUP-TF1 depletion impaired dormant phenotype in LA-exposed PCa cells which is supported by LA-driven lipid droplet (LD) accumulation. Blocking LD formation reduced COUP-TF1 expression and reversed dormancy traits. Through LD-derived acetyl moieties LA-exposed PCa cells increased H3K27 acetylation at the COUP-TF1 promoter, thereby regulating its expression. Therefore, histone acetylation reading (Bromodomain) inhibition prevents dormancy activation. Importantly, PTs displayed high LA levels, whereas bone and lung metastatic niches showed lower LA concentrations. LA washout reduced COUP-TF1 and restored proliferation, indicating that low-LA environments contribute to favor dormancy exit and metastatic outgrowth.

Conclusions High extracellular LA promotes a dormant, stem-like PCa cell state through an LD-BRD4-COUP-TF1 metabolic-epigenetic axis. Conversely, low LA levels in metastatic tissues facilitate proliferative reactivation. Targeting lactate metabolism, lipid storage, or BET activity may help prevent dormant cell selection and progression in Pca.

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Poster 37

β2-Microglobulin at the Crossroads of Amyloidosis and Multiple Myeloma: Biochemical and Cellular Perspective

Nour alhoda Kassab[1], Giulia Rizzi [2], Laure Gauthier-manuel [2] Cristina Visentin[1], Stefano Ricagno[1]

Department of Biosciences, Università degli Studi di Milano, Milan 20133, Italy
Affiliation 1; cristina.visentin@unimi.it
Affiliation 2; stefano.ricagno@unimi.it

Beta-2 microglobulin (β2m) is the light chain of the MHC class I complex and, under destabilizing conditions, can aggregate into amyloid fibrils associated with pathological states. Elevated circulating β2m levels are observed in multiple myeloma patients, where it contributes to disease progression. Within the tumor microenvironment, β2m is internalized by tumor-associated macrophages, accumulates in lysosomes, and promotes amyloid formation, contributing to activation of the NLRP3 inflammasome.

In this study, we investigated the effects of wild-type β2m and variants with different aggregation propensities on the THP-1 monocytic cell line. The selected variants exhibit distinct structural stability and amyloidogenic behaviour. D76N shows reduced stability and increased amyloid propensity; ΔN6 is a truncated and destabilized form; D59P exhibits enhanced amyloid formation; W60G shows reduced amyloidogenicity.

The recombinant proteins produced in our lab were used to test their effects on monocytic cell lines. Cellular viabilities were assessed using MTT assays, while inflammasome activation was evaluated by western blot and ELISA.

Treatment with β2m variants resulted in a time-dependent decrease in THP-1 cell viability, correlating with their aggregation propensity. Increased expression of NLRP3 and caspase-1 was detected, along with elevated secretion of pro-inflammatory interleukins.

These findings support that β2m aggregation promotes inflammasome activation and inflammatory signalling in macrophages, which may contribute to the progression of multiple myeloma within the tumour microenvironment.

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Poster 38

Erα Signaling Regulates Immune Escape and Response to Immunotherapy in NSCLC

Angelo Meoli[1,2], Sofia la Vecchia[1], Alberto Lipani[1,2], Ruslana Mollo[1,2], Luca Alessandrì[2,3], Maddalena Arigoni[2,3], Raffaele Calogero[2,3], Costanzo Costamagna[1,2], Iris Chiara Salaroglio[1,2], Chiara Riganti[1,2], **Joanna Kopecka**[1,2]

[1] Department of Oncology, University of Torino, Torino, Italy; joanna.kopecka@unito.it.
[2] Molecular Biotechnology Center "Guido Tarone", University of Torino, Torino, Italy.
[3] Department of Molecular Biotechnology and Life Sciences, University of Torino, Torino, Italy.

Background: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality worldwide. Although immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis have improved clinical outcomes, only a minority of patients achieve durable responses. We previously identified estrogen receptor alpha (ERα) as a regulator of PD-L1 expression and demonstrated that ERα inhibition with letrozole enhances the efficacy of pembrolizumab in NSCLC. Emerging evidence suggests that lipid metabolism may also influence anti-tumor immunity and response to ICIs.

Aim: To investigate the interplay between ERα signaling, lipid metabolism, and immune regulation in NSCLC, and to determine whether modulation of fatty acid metabolism can improve the efficacy of ICIs.

Results: Using ERα-high NSCLC cell lines, ChIP-seq and RT-qPCR analyses demonstrated that ERα regulates not only PD-L1 but also multiple immune- and metabolism-related genes. In particular, our analyses showed that ERα influences the expression of genes involved in immune regulation and lipid metabolism in NSCLC cells. Changes in lipid content led to altered membrane composition and reduced PD-L1/PD-1 interaction. Similar immune-sensitizing effects were observed following supplementation with the ω3-polyunsaturated fatty acid docosahexaenoic acid (DHA), which improved the efficacy of pembrolizumab in preclinical NSCLC models.

Conclusions: Our findings reveal a novel link between ERα signaling, lipid metabolism, and immune escape in NSCLC. Modulation of membrane lipid composition through ERα inhibition or PUFA supplementation enhances tumor sensitivity to ICIs. These results support the repurposing of letrozole and DHA as immune-metabolic adjuvants to improve immunotherapy responses in NSCLC patients.

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Poster 39

Uncoupled NRF2 activation and persistent unfolded protein response characterize Neutrophils in Myelofibrosis

Enrico La Spina[1], Cesarina Giallongo[1], Lucia Longhitano[1], Sebastiano Giallongo[3], Andrea Duminuco[2], Tatiana Zuppelli[1], Deborah Calvo[1], Angela Maria Amorini[1], Francesco Bellia[1], Giuseppe Conti[1], Giovanni Li Volti[1], Alessandro Lucchesi[4], Giuseppe A. Palumbo[2], Daniele Tibullo[1]

[1]Department of Biotechnological and Biomedical Sciences, University of Catania, Italy;
[2]Department of Medical, Surgical Sciences and Advanced Technologies G.F. Ingrassia, University of Catania, Italy;
[3]Department of Medicine and Surgery, University of Enna "Kore", Enna, Italy;
[4]IRST "Dino Amadori", Meldola, Italy; alessandro.lucchesi@irst.emr.it

Background: Myelofibrosis (MF) is characterized by chronic inflammation and oxidative stress involving the myeloid compartment. Pathologically activated neutrophils (PMNs) exhibit metabolic and functional alterations contributing to disease progression through mitochondrial dysfunction and oxidative stress.

Aim: To investigate the transcriptional and metabolic profile of circulating PMNs from MF patients before and after anti-JAK therapies commonly used in clinical practice, focusing on oxidative stress and unfolded protein response (UPR) pathways.

Results: PMNs from untreated MF patients showed increased mitochondrial ROS production, elevated mitochondrial mass, and mitochondrial membrane depolarization, indicating profound metabolic dysfunction. Single-cell RNA sequencing and metabolomic analyses revealed enrichment of inflammatory pathways, including TNF-α/NF-κB, IL6/JAK/STAT3, and interferon signaling, together with activation of UPR and oxidative phosphorylation programs. Although NRF2 (NFE2L2) expression was increased, its downstream target HMOX1 remained suppressed, suggesting ineffective antioxidant activation under chronic oxidative stress. This NRF2 uncoupling co-occurred with persistent UPR/ER-stress signaling, highlighting maladaptive proteostatic responses. Following anti-JAK therapies, inflammatory and interferon pathways were reduced and UPR signatures partially normalized. However, residual oxidative stress and persistent NRF2 activation without full HMOX1 restoration suggested incomplete metabolic reprogramming. Overall, PMNs in MF exhibited a dual stress phenotype characterized by NRF2-HMOX1 uncoupling and chronic UPR activation.

Conclusions: PMNs contribute to MF progression by sustaining a pro-inflammatory microenvironment. Oxidative and proteostatic rewiring represent key features of PMN dysfunction in MF and may constitute therapeutic targets beyond JAK inhibition.

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Poster 40

Role of primary cilia in targeted therapy resistance in melanoma

Pietro Lugato[1], Raul Castro-Portuguez[1], Ana de Souza[1], Lin Nan[1], Annalena Weber[1], Roxana Oberkersch[1], Massimo Santoro[1]

[1]Laboratory of angiogenesis and cancer metabolism, University of Padova, Department of Biology

Background: Melanoma is the most aggressive skin cancer, with activating BRAF mutations in over 50% of cases. Combined BRAF/MEK inhibition induces rapid tumor regression, but durable responses are limited by acquired resistance. A major driver is phenotypic plasticity, enabling non-genetic adaptation through coordinated transcriptional, signaling, translational, and metabolic reprogramming. While transcriptional and signaling mechanisms are well characterized, the role of selective mRNA translation and its interplay with metabolism remains unclear.

Aim: To define the functional interplay between amino acid metabolic rewiring, translational reprogramming, and primary cilia in drug-resistant melanoma, and identify targetable vulnerabilities.

Results: Drug-resistant (DR) models showed global translational repression and reduced mTORC1 activity, alongside selective translation of mRNAs linked to ciliogenesis (RiboLace). Increased ciliogenesis was confirmed in vitro and in vivo. Genetic inhibition of ciliogenesis enhanced sensitivity to therapy, supporting its functional role in resistance. Glutamine starvation induced ciliogenesis, linking cilia to amino acid metabolism. Ribo-seq codon occupancy analysis revealed reduced aspartate codon usage. Consistently, modulation of glutamine–aspartate metabolism promoted aspartate accumulation in DR cells, while Ift88 knockout impaired glutamine utilization, indicating coupling between ciliogenesis and metabolic rewiring.

Conclusions: MAPK inhibition induces global translational repression while enabling selective translation of a ciliogenesis program. This adaptive response is coupled to glutamine–aspartate–asparagine metabolic rewiring, potentially via amino acid–dependent control of codon-specific translation. This cilia-centered translational–metabolic axis represents a previously unrecognized mechanism driving phenotypic plasticity and drug resistance in melanoma.

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Poster 41

Loss of GLS1 promotes serine biosynthesis to sustain nucleotide production and proliferation in melanoma cells

Alessia Magro[1], Jair Gonzalez Marques Junior[2], Marco Fantuz[3], Marco Sciacovelli[4], Daniela de Zio[5], Alex Von Kriegsheim[2], Claudio Laquatra[1] and Andrea Rasola[1]

[1]Department of Biomedical Sciences, University of Padova, Padova, Italy; alessia.magro.1@studenti.unipd.it, claudio.laquatra@unipd.it, andrea.rasola@unipd.it
[2]Cancer Research UK Scotland Centre, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK; jmarques@exseed.ed.ac.uk, Alex.VonKriegsheim@ed.ac.uk
[3] Veneto Institute of Molecular Medicine (VIMM), Padova, Italy, marco.fantuz@vimm.it
[4]Department of Molecular and Clinical Cancer Medicine, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, UK; M.Sciacovelli@liverpool.ac.uk
[5]Institute of Molecular Medicine (IMM), University of Southern Denmark (SDU), Odense, Denmark. dzio@health.sdu.dk

Background: Melanoma is the most aggressive and treatment-resistant form of skin cancer and displays marked metabolic plasticity, largely driven by activation of the MAPK pathway following mutations such as BRAFV600E. Melanoma cells are known to rely on glutamine (Gln) metabolism to sustain tumor growth, promote metastatic potential, and acquire drug resistance. Although targeting Gln metabolism represents a promising therapeutic strategy, the high metabolic adaptability of melanoma cells makes drug-resistance a perpetual challenge.

Aim: This study investigates the adaptations of melanoma cells in which Gln metabolism was disrupted by knocking-out glutaminase 1 (GLS1), with the goal of identifying novel, therapeutically exploitable metabolic vulnerabilities.

Results: Using metabolomic, transcriptomic and *in vitro* tumorigenic analyses, we found that GLS1 loss in melanoma cells triggers a compensatory induction in the synthesis of glutamate, which is redirected toward *de novo* glutamine synthesis. Simultaneously, GLS1-deficient cells upregulate *de novo* serine synthesis and serine-to-glycine conversion, generating one-carbon units that fuel nucleotide biosynthesis and cell proliferation. Consistently, these cells accumulate intermediates of both purine and pyrimidine synthesis pathways, and pharmacological inhibition of serine hydroxymethyl transferase 2 (SHMT2), a key component of the Ser synthesis pathway, significantly impairs their tumorigenic potential.

Conclusions: My findings uncover a compensatory metabolic axis that emerges upon glutaminolysis impairment, positioning SHMT2 as a key regulator of the metabolic rewiring sustaining cancer cell growth. By establishing this pathway as an essential dependency, my results provide a compelling rationale for a synthetic lethality strategy opening actionable therapeutic opportunities for melanoma treatment.

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Poster 42

EXPLORING METABOLIC CROSSTALK IN GLIOBLASTOMA–MICROENVIRONMENT USING AN INNOVATIVE HUMAN TRI-CULTURE SYSTEM

Renata Mangione[1,2], Federica Blundo[1], Emiliano Maiani[1,2], Giuseppe Caruso[1,2], Angela Maria Amorini[3], Giuseppe Lazzarino[3], Barbara Tavazzi[1,2] , Giacomo Lazzarino[1,2]

[1]Departmental Faculty of Medicine, UniCamillus-Saint Camillus International University of Health Sciences, Rome, Italy;
[2]IRCCS San Camillo Hospital, Venice, Italy;
[3]Department of Biomedical and Biotechnological Sciences, University of Catania, Italy

renata.mangione@unicamillus.org

Background: Glioblastoma (GBM) is a highly aggressive primary brain tumor characterized by diffuse infiltration and poor prognosis, with limited response to current therapies. Increasing evidence indicates that the tumor microenvironment plays a central role in supporting GBM progression and therapy resistance.

Aim: This study aimed to investigate the metabolic and molecular crosstalk between GBM cells and surrounding brain cells by developing an in vitro tri-culture model that reproduces critical features of tumor–microenvironment interactions

Results: We established a human tri-culture system combining GBM cells with astrocytes, neurons, and microglia in physically separated compartments connected by a shared medium, allowing metabolic communication. Findings revealed that GBM cells induced pronounced metabolic perturbations in neighboring brain cells, affecting intracellular metabolic pathways and accompanied by measurable alterations in the extracellular milieu. These changes were consistently observed across distinct tri-culture configurations, indicating a dynamic metabolic interplay between tumor and non-tumoral cellular components.

Conclusions: This tri-culture platform represents a robust and versatile model for studying GBM–microenvironment interactions and highlights GBM-driven metabolic reprogramming as a key mechanism affecting non-tumoral brain cells. The system may support the identification of novel therapeutic targets aimed at disrupting tumor–TME crosstalk and limiting GBM progression.

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Poster 43

Genetic disruption of *FKBP5* alternative splicing enhances lymphocyte-mediated antitumor immunity

Laura Marrone[1], Valeria Di Giacomo[1], Gennaro Acanfora[2], Marialuisa Alessandra Vecchione[1], Elena Vigliar[2], Antonino Iaccarino[2], Nicola Russo[3] Emanuele Sasso[1], Guendalina Froechlich[1], Rosetta Abate[1], Simona Caggiano[1], Federica Aracri[1], Simona Romano[1] and Maria Fiammetta Romano[1]

[1]Department of Molecular Medicine and Medical Biotechnology, Federico II University, 80131, Naples, Italy
[2]Department of Public Health, Federico II University, 80131, Naples, Italy
[3]Animal models core facility Biogem S.c.ar.l., 83031, AV, Italy

Background: FKBP51 is a multifunctional immunophilin regulating key signaling pathways, including NF-κB, AKT, and steroid receptor trafficking. Alternative splicing of the *FKBP5* gene produces a shorter isoform, FKBP51s, lacking the C-terminal TPR domain required for protein–protein interactions. Emerging evidence suggests that *FKBP5* splicing may regulate T-lymphocyte expansion and immune homeostasis.

Aim: To determine whether the balance between canonical and spliced FKBP5 isoforms controls the magnitude and persistence of immune responses and influences antitumor immunity.

Results: We generated a knock-in mouse model (huFKBP5) expressing only the full-length human FKBP51 isoform, thereby eliminating endogenous splice variants. Homozygous huFKBP5+/+ mice were underrepresented, infertile, and displayed widespread lymphoid infiltrates, indicating disrupted immune homeostasis. In a syngeneic melanoma model, huFKBP5+/- mice exhibited enhanced antitumor responses, including reduced tumor growth, increased lymphocyte infiltration, elevated cytotoxic markers (perforin, Bax, cleaved caspase-3/7, gasdermin E), and upregulation of CCR7 and CXCR5 on tumor-infiltrating lymphocytes. Synthetic mRNA experiments confirmed a conserved role for FKBP51 in lymphocyte activation and identified FKBP51s as a dominant-negative regulator of murine lymphocyte effector function.

Conclusions: Blocking FKBP51 splicing preserves lymphocyte effector activity and limits exhaustion, thereby enhancing antitumor immunity and reducing tumor tolerance.

Poster 44

The CHK1 inhibitor prexasertib restores differentiation in ATRA-resistant acute promyelocytic leukemia cells by rewiring mitochondrial metabolism

Lucilla Martinelli[1§], Arianna Chilelli[1§], Maria Franza[1], Aurora Melfi[1], Victoriano Francisco Mulero Mendez[2], Alessandra di Masi[1]

[1]Dipartimento di Scienze, Università degli Studi Roma Tre, Viale Guglielmo Marconi 446, Roma, Roma, Italy; e-mails: luc.martinelli4@stud.uniroma3.it; ari.chilelli@stud.uniroma3.it; alessandra.dimasi@uniroma3.it
[2]Facultad de Biologia, Universidad de Murcia, Campus de Espinardo Edificio nº 20, Murcia, Murcia, Spain; e-mail: Vmulero@um.es

Background: Acute promyelocytic leukaemia (APL) is a subtype of acute myeloid leukaemia caused by the oncogenic PML-RARα fusion protein. While all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO) have significantly improved patient outcomes, resistance to these treatments remains a significant challenge, emphasising the need for new, low-toxicity differentiation therapies. We have identified CHK1, a nuclear serine/threonine kinase, as a promising therapeutic target in APL. Indeed, two CHK1-specific inhibitors, MK-8776 and prexasertib, have been shown to promote PML-RARα degradation and induce differentiation in ATRA-resistant APL cells (DOI: 10.1016/j.bcp.2023.115675).

Aim: To investigate the effects of prexasertib in ATRA-resistant APL cells, we combined metabolic profiling, differentiation assays and *in vivo* validation in zebrafish APL xenograft models.

Results: CHK1 inhibition triggered PML-RARα degradation and robust differentiation of ATRA-resistant APL cells. Mechanistically, CHK1 localised to the mitochondria, and the blockade of CHK1 impaired oxidative phosphorylation and ATP production through a SIRT3-dependent pathway. This identified CHK1 as a regulator of leukaemic metabolic fitness. Prexasertib also increased ROS production and synergized with ATO to enhance differentiation. In zebrafish APL xenografts, prexasertib reduced the burden of leukaemic cells and attenuated the associated immune response, thus supporting its *in vivo* efficacy.

Conclusions: These findings suggest that CHK1 inhibition is a promising strategy for the differentiation-based treatment of APL, targeting mitochondrial metabolism and overcoming ATRA/ATO resistance.

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Poster 45

Redox homeostasis is disrupted by flavonoids contained in bergamot juice in colorectal cancer

Alessandro Maugeri[1], Martina Farina[2] and Michele Navarra[2]

[1]Department of Veterinary Sciences, University of Messina (Italy); amaugeri@unime.it
[2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina (Italy)

Background: After lung cancer, colorectal cancer (CRC) is the second most prevalent cause of cancer-related mortality in the developed world. A diet high in flavonoids may reduce the risk of several types of cancer, including colorectal cancer.

Aim: The purpose of this study was to determine the potential anticancer effects of a flavonoid-rich extract of bergamot juice (BJe) in CRC models, assessing the underlying mechanisms.

Results: In this study, we observed that BJe inhibited the growth of several CRC cells, especially HCT-116. In this cell line, BJe was able to induce both early and late apoptosis, along with block cell cycle in G1 phase. This effect was coupled with a modulation of pro- and anti-apoptotic markers, as well as cell cycle-related factors. Examining the potential mechanisms, we observed that BJe was able to prompt the production of reactive oxygen species and, consequently, impair the mitochondrial membrane potential. Diving deeper into molecular events, we found that BJe was able to induce DNA damage as demonstrated by the increase of levels of the oxidized nucleobase 8-oxo-2'-deoxyguanosine, as well as phosphorylation of histone H2A.X, known marker of double-strand breaks. In the azoxymethane-induced murine model of sporadic CRC, oral administration of BJe was able to reduce the number of aberrant crypt foci, as well as the percentage of mice bearing both polyps and tumors, together with their number.

Conclusions: Our findings highlight the significance of BJe as a regulator of CRC cell death both in vitro and in vivo, enhancing our understanding of the underlying mechanism of action.

Poster 46

Dissecting the physical and functional interaction between RCC1L and NME4

Vittoria Michelotto[1], Carlotta Paoli[2], Francesca Bozzetto[3], Roberta Noè[4], Marco Fantuz[5], Beatrice Calciolari[6], Nicla Lorito[7], Elisa Giovannetti[8], Vincenzo Corbo[9], Ildiko Szabo[10], Andrea Morandi[11], Rushika Perera[12], Luca Scorrano[13], Alessandro Carrer[14]

[1]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; vittoria.michelotto@studenti.unipd.it; vittoria.michelotto@vimm.it;
[2]Department of Biology, University of Padova, Italy; carlotta.paoli@unipd.it
[3]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; francesca.bozzetto.1@studenti.unipd.it; francesca.bozzetto@vimm.it
[4]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; roberta.noe@studenti.unipd.it; roberta.noe@vimm.it;
[5]Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; marco.fantuz@vimm.it;
[6]Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; beatrice.calciolari@vimm.it;
[7]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Firenze, Italy; nicla.lorito@unifi.it
[8]Fondazione Pisana per la Scienza ONLUS, San Giuliano Terme (PI), Italy; elisa.giovannetti@gmail.com
[9]Department of Engineering for Innovation Medicine, University of Verona, Italy; vincenzo.corbo@univr.it
[10]Department of Biology, University of Padova, Italy; ildiko.szabo@unipd.it
[11]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Firenze, Italy; andrea.morandi@unifi.it
[12]Department of Anatomy, University of California San Francisco, San Francisco, CA, USA; rushika.perera@ucsf.edu
[13]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; luca.scorrano@unipd.it; luca.scorrano@vimm.it
[14]Department of Biology, University of Padova, Italy; Veneto Institute of Molecular Medicine (VIMM), Padova, Italy; alessandro.carrer@unipd.it; alessandro.carrer@vimm.it.

Background: The GTPase protein OPA1, which regulates inner mitochondrial membrane (IMM) fusion and cristae structure, is one of the most important players in mitochondrial dynamics. We speculate that two of its interactors, the nucleotide diphosphate kinase NME4 and the guanine nucleotide exchange factor RCC1L, elevate OPA1 activity in pancreatic ductal adenocarcinoma (PDAC). Interestingly, we previously found that RCC1L and NME4 are overrepresented in the mitochondrial proteome of PDAC cells compared to non-transformed pancreatic epithelial cells, and that high NME4 expression is associated to poor prognosis in human patients. This suggests that these two proteins play a role in PDAC progression, possibly through regulation of OPA1 function. Indeed, downregulation of either NME4 or RCC1L impairs mitochondrial network, ultrastructure, and mitochondrial respiration in PDAC cells lines, while OPA1 overexpression promotes pancreatic carcinogenesis in autochthonous mouse models.

Aim: Our work aims to dissect the physical interaction between RCC1L and NME4 and understand whether OPA1 activity depends on this protein nexus.

Results: Proximity ligation assay (PLA) showed that OPA1, NME4 and RCC1L interact in malignant and non-malignant pancreatic cells. Downregulation of RCC1L decreases NME4/OPA1 PLA foci, confirming the role of RCC1L in mediating NME4/OPA1 interaction. I observed little co-immunoprecipitation of RCC1L/NME4, suggesting a weak interaction between the two proteins. To further investigate the functional link between NME4 and OPA1, I analyzed the mitochondrial network of MEFs overexpressing NME4, observing that NME4 alone is not able to increase fusion.

Conclusions: Our data collectively support a model in which NME4 and RCC1L sustain OPA1 activity during PDAC progression, possibly through the remodeling of mitochondria ultrastructure. Our work identifies metabolic compartmentalization of nucleotide biosynthesis as a critical process for PDAC carcinogenesis.

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Poster 47

Integrated stress response and lipid remodeling are adaptive mechanisms triggered by Complex I deficiency in cancer cells

Sara Milioni[1], Manuela Sollazzo[2], Ivana Kurelac[3], Anna Carolina Emi De Lima Tanada[4], Laura Lanteri[2], Stefano Miglietta[5], Luigi D'Angelo[2], Monica De Luise[3], Francesca Nanetti[3], Gaia Tioli[6], Claudia Zanna[7], Federico Manuel Giorgi[2], Adriano de Britto Chaves Filho[8], Isaia Barbieri[1], Almut Schulze[8], Enrica Calura[4], Giuseppe Gasparre[3#], Luisa Iommarini[2*#], Anna Maria Porcelli[2*]

[1]University of Turin, Department of molecular biotechnology and health sciences, Molecular Biotechnology Center, Italy; sara.milioni@unito.it; isaia.barbieri@unito.it
[2]University of Bologna, Department of Pharmacy and Biotechnology, Italy; manuela.sollazzo2@unibo.it; laura.lanteri3@unibo.it; luigi.dangelo4@unibo.it; luisa.iommarini2@unibo.it; annamaria.porcelli@unibo.it; federico.giorgi@unibo.it
[3]University of Bologna, Department of Medical and Surgical Sciences, Italy; ivana.kurelac@unibo.it; monica.deluise2@unibo.it; francesca.nanetti2@unibo.it; giuseppe.gasparre3@unibo.it
[4]University of Padova, Department of Biology, Italy; annacarolinaemi.delimatanada@studenti.unipd.it; enrica.calura@unipd.it
[5]University of Bari, Department of Biosciences, Biotechnologies and Environment, Italy; stefano.miglietta@uniba.it
[6]IRCSS Bologna - Istituto delle Scienze Neurologiche, Italy: gaia.tioli@ausl.bologna.it
[7]University of Bologna, Department of Biomedical and Neuromotor Sciences, Italy; claudia.zanna@unibo.it
[8]Division of Tumor Metabolism and Microenvironment, German Cancer Research Center (DKFZ) and DKFZ-ZMBH Alliance, Heidelberg, Germany; adriano.debrittochavesfilho@dkfz-heidelberg.de; almut.schulze@dkfz-heidelberg.de

Background: We previously demonstrated that the lack of nuclear-encoded NDUFS3, a core subunit of CI, causes a profound metabolic reprogramming, confining cancer cells in a low proliferative status without a complete eradication of the tumor, likely due to the activation of adaptive survival pathways.

Aim: The aim of this project is to investigate the molecular and metabolic pathways triggered by CI dysfunction to sustain cell survival.

Results: We exploited multiple cancer cell lines of different tissue origins, in which we knocked out NDUFS3. We incubated cells in low glucose medium to enhance the bioenergetic impairment and to better mimic the nutrient-deprived tumor microenvironment. NDUFS3$^{-/-}$ cells show mitochondrial depolarization, which in turn triggers the activation of the mitochondrial protease OMA1 leading to the processing of its targets, including those responsible for network fragmentation. These mitochondrial alterations are accompanied by a consistent activation of Integrated Stress Response (ISR) as proved by the phosphorylation of eIF2α, the block of protein synthesis, the nuclear re-localization of ATF4 and the increased expression of CHOP. These results are also observed *in vivo* and upon pharmacological CI inhibition by using EVP 4593. ISR is responsible for survival of CI-defective cells and is directed by OMA1 and suppressed when the protease expression is prevented. Glucose starvation also triggers the accumulation of LDs in close proximity to deranged mitochondria of CI-defective cells both *in vitro* and *in vivo*, accompanied by a profound alteration of lipid content.

Conclusions: Loss of CI and glucose restriction trigger OMA1-mediated ISR to overcome the stress status and survive.

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Poster 48

S-Adenosylmethionine Potentiates Cabazitaxel Activity in Metastatic Castration-Resistant Prostate Cancer

Laura Mosca[1], Roberta Arpino[2], Francesca Cadoni[2], Cristina Pagano[3], Marina Porcelli[2]

[1]Department of Human Sciences and Promoting of the Quality of Life, San Raffaele University, Rome, Italy; laura.mosca@uniroma5.it
[2]Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy; roberta.arpino@unicampania.it; francesca.cadoni@unicampania.it; marina.porcelli@unicampania.it;
[3]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; cristina.pagano@unina.it;

Background: Metastatic castration-resistant prostate cancer (mCRPC) represents the most aggressive form of prostate cancer (PCa). Given the limited effectiveness of conventional taxane-based therapies, cabazitaxel (CBZ), a second-generation semi-synthetic taxane, has been used. Although CBZ has shown efficacy in patients resistant to commonly utilized taxanes such as docetaxel and paclitaxel, and is currently employed as a second-line treatment for mCRPC, its clinical use is constrained by significant adverse effects and the progressive development of drug resistance, which remains a major cause of therapeutic failure. In this context, the use of naturally derived adjuvants in chemotherapy is gaining increasing attention as a promising research strategy. S-Adenosyl-L-methionine (AdoMet) is an endogenous sulfur-containing nucleoside that functions as the principal methyl donor for a wide range of methyltransferases, playing a central role in numerous essential cellular biochemical processes.

Aim: In the present study, we investigated the therapeutic potential of combining AdoMet with CBZ in mCRPC cells.

Results: Experiments conducted on DU145 and PC-3 cell lines revealed that the combined treatment was more effective in inhibiting cancer cell proliferation compared to either agent administered alone. This synergistic effect appears to be associated with enhanced production of reactive oxygen species (ROS) and impairment of the cellular antioxidant defense system, as evidenced by decreased levels of glutathione, GPX4, and catalase. The resulting oxidative stress led to DNA damage and disruption of mitotic spindle formation, ultimately triggering cell cycle arrest and apoptosis.

Conclusions: Overall, these findings suggest that AdoMet can enhance the antitumor activity of CBZ in mCRPC cells, thereby improving therapeutic efficacy.

Poster 49

Metabolic and Lipidomic Remodeling during Lung Cancer Progression

Marta Nobile[1], Simone Serrao[1], Benedetta Gerosa[2], Eleonora Bossi[1], Ilaria Alberico[2], Giulia Bertolini[2] and Giuseppe Paglia [1]

[1]University of Milano Bicocca, Department of Medicine and Surgery, Via Raoul Follereau 3, 20854, Vedano al Lambro (MB), Italy; m.nobile13@campus.unimib.it, simone.serrao@unimib.it, e.bossi13@campus.unimib.it, giuseppe.paglia@unimib.it
[2]Fondazione IRCCS Istituto Nazionale dei Tumori, Unit of Epigenomics and Biomarkers of Solid Tumors, Via Venezian 1, 20133, Milano, Italy; Benedetta.Gerosa@istitutotumori.mi.it, Giulia.Bertolini@istitutotumori.mi.it, Ilaria.Alberico@istitutotumori.mi.it

Background: Lung cancer is the leading cause of cancer-related mortality worldwide, mainly due to metastatic dissemination and late diagnosis. Circulating tumor cells (CTCs), released from the primary tumor into the bloodstream, represent a key intermediate stage in metastasis. However, the metabolic adaptations supporting tumor dissemination and metastatic colonization remain poorly understood.

Aim: This study investigated metabolic remodeling during the transition from primary tumor (PT) to CTCs and metastasis (Met) in non-small cell lung cancer (NSCLC). PT, CTC and Met cell lines were generated from a patient-derived xenograft model by isolating cells from the primary tumor, blood, and lungs, respectively. Cells were expanded in vitro and analyzed through untargeted mass spectrometry-based metabolomics and lipidomics.

Results: Distinct metabolic signatures emerged across tumor stages. The transition from PT to CTCs was characterized by alterations in purine metabolism, glycolysis, branched-chain amino acid catabolism and fatty acid β-oxidation. CTCs also showed increased accumulation of storage lipids, suggesting adaptive mechanisms supporting survival in circulation. Met cells displayed a lipid metabolic switch, with increased storage and membrane lipids, consistent with enhanced membrane remodeling and invasive potential.

Conclusions: Our findings reveal dynamic metabolic and lipidomic remodeling during NSCLC evolution and identify CTCs as a metabolically adaptive cell population. These results provide insight into metabolic vulnerabilities associated with metastatic dissemination and may support novel therapeutic strategies targeting lung cancer progression.

Poster 50

The miR-155/MALAT-1 ceRNA axis controls mitochondrial metabolism and chemoresistance in lung cancer

Martina Perin[1,2], Niloufar Jafari[1,2], Simona Fontana[1,2], Valeria Ramundo[1,2], Iris Chiara Salaroglio[1,2], Francesca Orso[1,3], Daniela Taverna[1,4], Joanna Kopecka[1,2], Chiara Riganti[1,2]

[1]Molecular Biotechnology Center "Guido Tarone", University of Turin, Turin, Italy. martina.perin@unito.it
[2]Department of Oncology, University of Turin, Turin, Italy.
[3]Department of Translational Medicine, University of Western Piedmont, Novara, Italy.
[4]Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy.

Background: CEBP/β, a transcription factor highly expressed in lung cancer has two isoforms: LAP and LIP. We previously showed that LAP induces a metabolic rewiring toward oxidative phosphorylation (OXPHOS) and chemoresistance in non-small cell lung cancer (NSCLC), while LIP has the opposite effects[1].

Aim: Our aim is to identify the upstream regulators of CEBP/β splicing and how they impact lung cancer metabolism and chemoresistance.

Results: By *in silico* analysis, we identified two ncRNAs predicted to interact with CEBP/β: MALAT-1 and miR-155 that are competing endogenous RNA (ceRNAs). Human NSCLC chemoresistant cell lines had LAP^{high}MALAT-1^{high}miR-155^{low} phenotype, high drug efflux transporters ABCB1 and ABCC1, high OXPHOS and ATP production that fuels ABC transporters. Moreover, they had a high saturated/poly-unsaturated fatty acid (SFA/PUFA) ratio that supported OXPHOS Chemosensitive cells had LAPlowMALAT-1lowmiR-155high phenotype, low drug efflux transporters ABCB1 and ABCC1, low SFA/PUFA ratio, OXPHOS and ATP production. Antisense oligonucleotide (ASO) MALAT-1 or miR155-mimic reversed LAP/LIP ratio and SFA/PUFA ratio, reduced OXPHOS metabolism, ABC transporters activity and chemoresistance. Conversely, MALAT1-overexpression or anti-miR155 produced the opposite phenotype. The supplementation with the PUFA docosahexaenoic acid (DHA) synergized with ASO-MALAT-1 in decreasing OXPHOS and chemoresistance in LAPhighcells and tumors.

Conclusions: MALAT-1/miR155 axis controls the levels of the CEBP/β LAP/LIP isoforms, impacting on metabolic phenotype and chemoresistance. Combination strategies based on MALAT-1-targeting agents and metabolic-targeting agents are promising chemosensitizing approaches in resistant NSCLC.

References: Salaroglio IC et al, J Exp Clin Cancer Res 2022; doi:10.1186/s13046-022-02447-6

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Poster 51

Mutant p53 drives glycolytic rewiring and protein Lactylation in Pancreatic Ductal Adenocarcinoma

Filippo Piccoli[1], Adriana Celesia[1], Alessia Cardile[1], Maria Poles[1], Federica Danzi[1], Alessandra Fiore[1], Massimo Donadelli[1]

[1]Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biochemistry, University of Verona, Verona, Italy. Email: filippo.piccoli@univr.it

Background: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies. A defining molecular feature of PDAC is the high prevalence of *TP53* mutations, that lead to the accumulation of a dysfunctional yet stable mutant p53 protein. Beyond loss of tumor suppressor activity, mutant p53 acquires oncogenic gain-of-function properties that actively promote tumor progression.

Aim: Emerging evidence suggests that one critical aspect of mutant p53 biology is its ability to reprogram cellular metabolism to support the energetic and biosynthetic demands of rapidly proliferating cancer cells. The overall goal of this project is to assess the contribution of mutant p53 in promoting PDAC progression through the regulation of lactylation.

Results: We generated *TP53* knockout (KO) clones in human PDAC cell lines harboring endogenous missense mutant p53: PANC-1, T3M4, and MIA PaCa-2, respectively harboring R273H, Y220C, R248W mutations. Bioenergetic profiling using ATP rate assays and glycolysis stress tests revealed a clear metabolic shift: cells expressing mutant p53 exhibit significantly increased glycolytic activity compared to their *TP53* KO counterparts. Our previous evidence demonstrated that mutant p53 stimulates lactate secretion from PDAC cells, rendering them particularly sensitive to treatment with glycolysis inhibitors and non-metabolizable glucose analogs, such as 2-deoxyglucose. Consistently, we observed modulation of LDHA expression, with higher levels in parental mutant p53-expressing cells compared to *TP53* KO clones, supporting enhanced conversion of pyruvate to lactate. Notably, this metabolic phenotype correlates with increased protein lactylation.

Conclusions: Our findings indicate that mutant p53 promotes a glycolytic and lactate-producing phenotype in PDAC cells, associated with increased protein lactylation and may contribute to epigenetic and functional remodeling during tumor progression. These results support the existence of a mechanistic link between mutant p53 and lactylation.

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Poster 52

Cancer-associated adipocytes (CAAs) transcriptional and epigenetic heterogeneity in triple-negative breast cancer via lipid-dependent reprogramming

Teresa Rossi[1], Elisa Salviato[1], Christian Boni[1], Egidio Iorio[2], Elisa Gasparini[3], Evelina Begnini[4], Coiro Saverio[4], **Mariaelena Pistoni**[1]

[1]Laboratory of Translational Research, AUSL-IRCCS of Reggio Emilia, Reggio Emilia, Italy;
[2]High Resolution NMR Unit, Core Facilities, Istituto Superiore di Sanità, Rome, Italy;
[3]Medical Oncology, Comprehensive Cancer Centre, AUSL-IRCS of Reggio Emilia, Italy;
[4]Breast Surgery Unit, AUSL-IRCCS of Reggio Emilia, Reggio Emilia, Italy;

Background: Triple-negative breast cancer (TNBC) is an aggressive, heterogeneous subtype with poor prognosis. Cancer-associated adipocytes (CAAs) in the tumor microenvironment modulate tumor progression, but their role in driving transcriptional and chromatin heterogeneity remains unclear.

Aim: Define how CAAs rewire the TNBC transcriptome and epigenome, promoting lipid-driven aggressiveness and heterogeneity, and identify potential biomarkers to reduce tumor progression. MDA-MB231 and BT549 cells were co-cultured with patient-derived CAAs isolated proximally to tumor lesions.

Results: CAAs co-culture induced a shared stem-like/mesenchymal and inflammatory phenotype, with decrease of cholesterol homeostasis and interferon responses, and upregulation of EMT and invasion pathways. Fatty acids released by CAAs were central to this rewiring, driving metabolic addiction to exogenous lipids. However, cell-line-specific adaptations emerged: MDA-MB231 showed a stronger inflammatory/cytokine response, and BT549 showed greater proliferation and migration. Then, merging DEGs from RNA-seq to ATAC-seq revealed chromatin remodeling in nucleosome-free regions in about 500 genes. Importantly, CAAs amplified this intrinsic transcriptional heterogeneity, with distinct metabolic strategies (immediate lipid oxidation in MDA-MB231 vs. lipid storage in BT549). This adipocyte-driven heterogeneity suggests that targeting common lipid-dependent pathways could reduce tumor diversity.

Conclusions: CAAs rewire TNBC cells toward a lipid-driven, aggressive phenotype by releasing fatty acids that reshape transcription and chromatin, sustaining tumor heterogeneity. Identifying biomarkers of lipid-dependent reprogramming may help stratify patients and reduce heterogeneity, offering a therapeutic strategy against CAA-TNBC crosstalk.

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Poster 53

Mutant p53 affects the mitochondrial proteome, promoting mitochondrial fragmentation and OXPHOS in pancreatic ductal adenocarcinoma cells

Maria Poles[1], Raffaella Pacchiana[1] Chiara Mortali[1], Barbara Cisterna[1], Nidula Mullappilly[2], Adriana Celesia[1], Federica Danzi[1], Martina Gaspari[3], Daniela Cecconi[1], Massimo Donadelli[1] and Alessandra Fiore[1]

[1]University of Verona, Strada Le Grazie 8, 37134, Verona, Italy
[2]School of Medicine, Duke University, Durham, North Carolina, USA.
[3]Utah Tech University, St. George, Utah, USA.

Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer marked by poor prognosis and frequent gain-of-function mutations in the *TP53* tumor suppressor gene. Wild-type p53 is a critical regulator of mitochondrial homeostasis, governing both glycolysis and oxidative phosphorylation to control cellular fate. Disruption of this regulatory role through p53 mutation impairs mitochondrial metabolism, likely contributing to cancer progression.

Aim: This project aims to investigate the impact of mutant p53 on mitochondrial biology and function, as understanding this relationship may provide new strategies to selectively overcome drug resistance and improve therapeutic outcomes in PDAC patients.

Results: We characterized the mitochondrial proteome of PDAC cells carrying the gain-of-function R273H *TP53*mutation, identifying a significant mutant p53-dependent upregulation of myosin heavy chain 14 (MYH14), a non-muscle myosin involved in mitochondrial dynamics. We then investigated how mutant p53 regulates mitochondrial architecture and functionality in PDAC cells. Using transmission electron microscopy and three-dimensional confocal imaging, our morphological and morphometric analyses showed that mutant p53 promotes mitochondrial fragmentation, while wild-type p53 favors mitochondrial elongation. Notably, this fragmented mitochondrial morphology correlates with elevated mitochondrial respiration and enhanced cristae efficiency.

Conclusions: These findings suggest that mutant p53 isoforms drive mitochondrial fragmentation through a mechanism involving MYH14, enhancing oxidative phosphorylation that may support PDAC cell growth and aggressiveness.

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Poster 54

Treatment with inhibitors of nicotinamide N-methyltransferase enzyme to reduce the aggressiveness of radioresistant haed and neck cancer cells

Veronica Pompei[1], Eleonora Gerini [1], Vita Petrone [2], Rossella Chirico [2], Matthijs J. van Haren[3], Nathaniel I. Martin[3], Roberto Campagna[4], Valentina Pozzi[1], Ira-Ida Skvortsova[5], Claudia Matteucci[2] and Davide Sartini[1]

[1]Department of Clinical Sciences, Polytechnic University of Marche, Ancona, Italy; v.pompei@staff.univpm.it; ele.gerini@gmail.com; v.pozzi@univpm.it; d.sartini@univpm.it
[2]Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy; vita.petrone01@gmail.com; chirico.rossella3@gmail.com; matteucci@med.uniroma2.it
Institute of Biology Leiden, Leiden University, Leiden, The Netherlands; m.vanharen@cantonitx.nl; n.i.martin@biology.leidenuniv.nl
[3]Department for the Promotion of Human Science and Quality of Life, San Raffaele Roma University, Rome, Italy; roberto.campagna@uniroma5.it
[4]Department of Therapeutic Radiology and Oncology, Medical University of Innsbruck, Innsbruck, Austria; ira.skvortsova@i-med.ac.at

Background. Head and neck squamous cell carcinoma (HNSCC) is characterized by high mortality rate, due to late-stage diagnosis and frequent resistance to radio- and chemotherapy. The overexpression of nicotinamide N-methyltransferase (NNMT) was observed in several solid tumours, promoting the aggressiveness of cancer cell. Based on this evidence, the role of NNMT as potential HNSCC therapeutic target deserves to be explored.

Aim. The involvement of NNMT in aggressiveness of HNSCC cell was speculated in parental (PA) and radioresistant (RR) subpopulations of FaDu and CAL-27 cell lines, respectively obtained from hypopharyngeal and tongue squamous cell carcinoma. In particular, NNMT and stem marker expression, as well as proliferation, viability, migration, sensitivity to chemotherapeutic drugs and response to treatment with NNMT inhibitors were analysed.

Results. Radioresistant clones of both cell lines showed an increased expression of OCT4 and NANOG stem markers compared to the parental counterparts, being associated to an upregulation of NNMT. The higher aggressiveness of RR cells compared with that of PA counterparts was demonstrated by enhanced viability, proliferation, migration, clonogenic capability, as well as reduced chemosensitivity. In RR cells, PARPs and SIRT1 activity was significantly higher compared to that of PA populations. Interestingly, treatment with NNMT inhibitors markedly decreased viability of RR cells compared to PA clones. Moreover, the inhibition of NNMT induced a lower clonogenic and migratory ability, as well as a reduced activity of SIRT1 and PARPs in RR cells.

Conclusions. These data demonstrate that NNMT plays a crucial role in radioresistance and aggressiveness in HNSCC cell lines. Therefore, the catalytic inhibition of the enzyme could represent a promising strategy for the development of an effective anti-cancer therapy for counteracting resistance to conventional HNSCC treatments.

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Poster 55

Exploiting glutamine metabolism to sensitize Triple-Negative Breast Cancer Cells to ATR Inhibition

Marilena Pontoriero[1], Anam Aftab[1], Marina La Chimia[1], Filippo Santise[1], Alessia Noto[1], Alessandra De Santis[1], Ferdinando Chiaradonna[2], Domenica Scumaci[1]

[1]Department of Experimental and Clinical Medicine, University of Magna Græcia, Catanzaro, Italy;
[2]Department of Biotechnology and Biosciences, University of Milano Bicocca, Milan, Italy. m.pontoriero@unicz.it

Background: Triple-negative breast cancer (TNBC) is an aggressive and heterogeneous breast cancer subtype frequently associated with *BRCA1* mutations and metabolic reprogramming, particularly a marked glutamine dependency[1,2]. Increasing evidence highlights a functional crosstalk between altered tumor metabolism and the DNA damage response (DDR), wherein nucleotide and amino acid metabolism intricately modulate ATR kinase signaling and replication stress tolerance[3].

Aim: Addressing the metabolic heterogeneity of TNBC, this study aimed to investigate the distinct glutamine metabolism profiles in *BRCA1*-mutated models and to evaluate whether pharmacological inhibition of glutaminase (GLS) synergistically enhances sensitivity to ATR inhibitors.

Results: Comparative proteomics revealed distinct molecular profiles between the two models: truncated HCC1937 cells overexpress stemness markers (CD9) and glutamine-utilizing enzymes (GLS, IDH3B), whereas the L1780P variant favors lipid (FASN) and nucleotide (ATIC) biosynthesis. Based on these findings, we targeted the specific glutamine dependency of HCC1937 cells to induce metabolic stress and sensitize them to ATR inhibition. Combined inhibition of GLS and ATR significantly compromised cell viability and led to the accumulation of DNA damage, demonstrating that metabolic rewiring directly modulates DDR susceptibility.

Conclusions: These findings highlight the pivotal role of BRCA1 mutation status in driving metabolic diversity within TNBC. By simultaneously targeting glutamine metabolism and ATR signaling, we achieved a synergistic antitumor effect, particularly in glutamine-dependent TNBC models. This study underscores the potential of metabolic stratification to improve patient outcomes, supporting the shift toward more tailored and effective therapeutic strategies in TNBC.

1. Guo S, Addison CL. Metabolic Vulnerabilities as a Therapeutic Target in Breast Cancer. *Curr Oncol.* 2026;33(2):129.
2. Fu X, et al. BRCA1 and Breast Cancer: Molecular Mechanisms and Therapeutic Strategies. *Front Cell Dev Biol.* 2022;10:813457.
3. Chatzidoukaki O, et al. DNA Damage Response and Metabolic Reprogramming in Health and Disease. *Trends Genet.* 2020;36(10):748-760

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Poster 56

Assessment of Urinary cfDNA Integrity Index (Alu 247/115) as a Potential Non-Invasive Biomarker for Bladder Cancer Detection

Valentina Pozzi[1], Roberto Campagna[2], Davide Sartini[1], Veronica Pompei[1], Angelo Cormio[1], Giulio Milanese[1], Andrea Benedetto Galosi[1], Monica Emanuelli[1] and Monia Cecati[2]

[1]Department of Clinical Sciences, Polytechnic University of Marche, Ancona, 60020, Italy; v.pozzi@staff.univpm.it; d.sartini@univpm.it; v.pompei@staff.univpm.it; a.cormio@pm.univpm.it; g.milanese@univpm.it; a.b.galosi@univpm.it; m.emanuelli@univpm.it
[2]Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University, 00166 Rome, Italy; roberto.campagna@uniroma5.it; monia.cecati@uniroma5.it

Background: Bladder cancer (BC) remains one of the most frequently diagnosed urological malignancies and is associated with a high rate of recurrence, requiring repeated diagnostic evaluations and prolonged follow-up. Conventional diagnostic tools, such as cystoscopy and urine cytology, present significant limitations due to their invasiveness, high costs, and suboptimal sensitivity, particularly in low-grade disease. The search for accurate and minimally invasive biomarkers is therefore of major clinical interest.

Aim: In this pilot study, we investigated urinary cell-free DNA (cfDNA) concentration and cfDNA integrity in 30 patients diagnosed with BC undergoing transurethral resection of bladder tumor and 20 healthy subjects. Quantitative Real-time PCR targeting Alu repetitive sequences (Alu 115 and Alu 247) was used to evaluate cfDNA abundance, while the cfDNA integrity index was determined by calculating the Alu 247/115 ratio.

Results: Patients with BC showed a significantly increased urinary cfDNA integrity index compared with healthy controls. Receiver operating characteristic (ROC) analysis demonstrated that the cfDNA integrity index achieved the highest diagnostic accuracy among the evaluated parameters, with an area under the curve (AUC) of 0.94 (95% CI: 0.876–0.998). Using an optimal threshold value of 1.37, the assay reached 86.7% sensitivity and 85.0% specificity.

Conclusions: These preliminary findings support the potential role of urinary cfDNA integrity assessment as a non-invasive biomarker for BC detection and surveillance. Larger prospective studies are needed to further confirm its clinical applicability.

Poster 57

Dimethyl fumarate and cisplatin synergistically induce ferroptosis and apoptosis in cervical cancer via glutathione depletion and p53 reactivation

Carolina Punziano[1], Giuseppina Minopoli[1], Simona Romano[1], Laura Marrone[1], Katia Aquilano[2], Maria Lina Tornesello[3], Raffaella Faraonio[1]

[1]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Italy.
[2]Department of Biology, University of Rome Tor Vergata, Italy.
[3]Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS Fondazione G. Pascale, Naples, Italy.

Background: Persistent infection with human papillomavirus (HPV) represents the main cause of cervical cancer. HPV-driven carcinogenesis is mediated by viral oncoproteins that interact with host factors, including p53, thereby disrupting pathways involved in tumor suppression and cancer progression. Cisplatin-based chemotherapy remains the standard treatment for cervical cancer. Unfortunately, therapeutic resistance and treatment failure are frequently observed, underscoring the need for novel therapeutic approaches. Dimethyl fumarate (DMF), an FDA-approved anti-inflammatory drug, has recently emerged as a promising anticancer agent capable of reducing tumor growth and metastasis.

Aim: We explored the antitumor effect of DMF in cervical cancer. Specifically, we tested if DMF alone (at high doses) provokes cervical cancer cell death or potentiates cisplatin efficacy when used at sub-cytotoxic doses.

Results: Our results showed that DMF at 200μM induces ferroptosis in cervical cancer cells and inhibits growth of spheroid models. This was mediated by impairment of the Nrf2-SLC7A11-dependent de novo glutathione (GSH) synthesis. In addition, cotreatment with sub-cytotoxic doses of both DMF (100 μM) and cisplatin (20 μM) significantly reduced cell viability and spheroid size compared with either treatment alone. Mechanistically, DMF/CDDP treatment favored p53 (re)activation, leading to SLC7A11 repression and triggering both ferroptosis and apoptosis.

Conclusions: Collectively, our data demonstrated that in this cancer context, GSH depletion achieved either by impaired Nrf2-SLC7A11 activation (with DMF at 200 μM alone) or by SLC7A11 repression caused by (re)activated p53 (under combined DMF plus CDDP treatments) may have relevant therapeutic implications.

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Poster 58

Methionine starvation and chaetocin combination therapy in colon cancer: mechanistic insights into cell death and adaptive responses

Samanta Raboni[1], Serena Montalbano[2], Simone Sidoli[3], Stefano Bettati[4] and Annamaria Buschini[2]

[1]Department of Food and Drug, University of Parma; samanta.raboni@unipr.it
[2]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma; serena.montalbano@unipr.it; annamaria.buschini@unipr.it
[3]Department of Biochemistry, Albert Einstein College of Medicine, USA; simone.sidoli@einsteinmed.edu
[4]Department of Medicine and Surgery, University of Parma; stefano.bettati@unipr.it

Background: Among targeted approaches exploiting cancer-specific metabolic vulnerabilities, the dependence of cancer cells on exogenous methionine prompted the use of methionine γ-lyase (MGL), a bacterial enzyme degrading methionine and inhibiting cancer growth through methionine starvation. Previous studies demonstrated that MGL treatment induces cytotoxicity, chromatin deregulation and adaptive stress responses in colon cancer cells, suggesting that combination strategies targeting compensatory survival pathways may potentiate the antitumor effect of metabolic starvation. In this context, chaetocin, a reported methyltransferase inhibitor potentially reinforcing MGL-induced epigenetic deregulation and interfering with adaptive responses, emerged as a promising combinatorial partner.

Aim: We investigated the molecular response of HT29 colon cancer cells to MGL-mediated methionine starvation, focusing on oxidative stress and cell death pathways, and evaluated the rationale of combining MGL treatment with chaetocin.

Results: MGL-induced methionine deprivation triggered early oxidative stress, followed by cell cycle arrest and activation of autophagy as a transient adaptive response. Under prolonged metabolic stress, ferroptosis emerged as the predominant cell death mechanism. These mechanistic findings suggested that cancer cells activate compensatory survival pathways to cope with methionine depletion. On this basis, the combination with chaetocin was explored for its potential to interfere with adaptive methionine recycling and epigenetic stress-response mechanisms.

Conclusions: These findings highlight that defining the mechanisms of cell death induced by methionine starvation is crucial for the rational development of combination therapies. Targeting adaptive responses emerging during metabolic stress through the integration of MGL-mediated methionine depletion and chaetocin may enhance antitumor efficacy in colon cancer cells.

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Poster 59

Patient-Derived 3D Chondrosarcoma Spheroids Reveal Cell Line-Dependent Sensitivity to Licochalcone A

Raspadori E.[1], Rossi M.[1], Anconelli L.[1], Rossi F.[1], Cappadone C.[1], De Biase D.[1,2], De Vita A.[3], Vanni S.[3], Violani S.[3], Miserocchi G[3], Malucelli E.[1], Iotti S.[1]

[1] Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy
[2]Solid Tumor Molecular Pathology Laboratory, IRCCS Azienda Ospedaliero- Universitaria di Bologna, Bologna, Italy
[3] Preclinic and Osteoncology Unit, Bioscience Laboratory, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, FC, Italy.

Background: Chondrosarcoma (CS) is a primary malignant bone tumor characterized by cartilaginous matrix and marked resistance to conventional chemo- and radiotherapy [1]. Licochalcone A (Lic-A), a natural chalcone from Glycyrrhiza species, has shown antitumor activity in sarcoma, including selective inhibition of IDH1-R132C, reduced 2-HG production, and induction of cell-cycle arrest and apoptosis [2]. This is relevant in CS, where IDH1/2 mutations promote D-2-hydroxyglutarate accumulation, epigenetic dysregulation and tumor progression [1]. Since CS behavior is also influenced by the bone marrow niche, 3D models reproducing tumor architecture and microenvironmental crosstalk are needed [3].

Aim: This study aimed to establish a 3D heterotypic co-culture model integrating patient-derived CS cell lines and bone marrow-derived mesenchymal stromal cells (BM-MSCs), to mimic the spatial and biological complexity of the sarcoma tumor microenvironment and assess treatment response in relation to IDH1 status.

Results: Four patient-derived CS cell lines were co-cultured with BM-MSCs to generate 3D spheroids by the liquid overlay technique. Stable heterotypic spheroid formation was achieved across all models, with cell line-dependent differences in growth kinetics. After spheroid maturation, Lic-A induced heterogeneous, dose-dependent antitumor responses. Dose-response analyses showed IC50 values ranging from 50 to 110 μM, depending on the CS cell line, indicating variable sensitivity to Lic-A in the 3D co-culture setting. NGS analysis was performed to define IDH1 mutational status and correlate genotype with treatment response.

Conclusion: The integration of 3D co-culture models with NGS-based profiling provides a relevant platform to study CS in a bone-like microenvironment. This approach may support the hypothesis that IDH1 mutations and tumor-stroma interactions represent key determinants of LicA response and may constitute a promising multi-target therapeutic strategy in CS.

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Reference

1. Landuzzi L, Ruzzi F, Lollini PL, Scotlandi K. Chondrosarcoma: New Molecular Insights, Challenges in Near-Patient Preclinical Modeling, and Therapeutic Approaches. Int J Mol Sci. 2025 Feb 12;26(4):1542.
2. He Z, Huang H, Fang J, Liu H, Yao X, Wu H. From 2D to 3D: transforming malignant bone tumor research with advanced culture models. J Zhejiang Univ Sci B. 2025 Nov 19;26(11):1059-1075.
3. Hu C, Zuo Y, Liu J, Xu H, Liao W, Dang Y, Luo C, Tang L, Zhang H. Licochalcone A suppresses the proliferation of sarcoma HT-1080 cells, as a selective R132C mutant IDH1 inhibitor. Bioorg Med Chem Lett. 2020 Jan 15;30(2):126825

Poster 60

Innovative Design of Raft-like Nanostructured Lipid Carrier for Nucleic Acid Delivery

Davide Ferrario[1], Sara Marelli[1], Paulo Siani[2], Frigerio G[2], Silvia Sesana[1], Cristiana Di Valentin[2] and **Francesca Re**[1]

[1]School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy; francesca.re1@unimib.it
[2]Department of Materials Science, University of Milano-Bicocca, Milano, Italy;

Background: Gene therapy is an emerging therapeutic strategy based on the delivery or modulation of genetic material. The clinical success of mRNA vaccines and RNA interference (RNAi)-based therapeutics has highlighted the potential of nucleic acid medicines (PMID:41890655; PMID:41476373). However, their application is limited by poor stability and inefficient cellular uptake. Lipid-based systems, particularly nanostructured lipid carriers (NLCs), have emerged as promising non-viral vectors due to their scalability, high loading capacity, and controlled release properties (PMID:38089666).

Results: Using molecular dynamics simulations, NLCs were rationally designed to form an imperfect lipid matrix containing cationic lipid nanodomains, enabling efficient loading of both siRNA and mRNA through enhanced electrostatic interactions while preserving endosomal escape capability. This composition allows the formation of cationic lipid rafts reconstituted in NLCs, useful to increase the loading efficiency of siRNA and mRNA and the transfection rate, keeping the endosomal escape feature useful for nucleic acid release. Importantly, by clustering positive charges within NLCs allows the same electrostatic interactions with nucleic acid achievable in conventional cationic LNPs, but with a lower overall content of cationic lipids in the formulation. This design improves biocompatibility and reduces cytotoxicity without compromising delivery efficiency.

Conclusions: The results provide a strong foundation for further studies to improve understanding of the stability, bioavailability, safety, and efficacy of these innovative RNA delivery nanoparticles *in vitro* and *in vivo*.

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Poster 61

Glutamate-Oxaloacetate Transaminase 2 (GOT2)-dependent aspartate availability confers ferroptosis resistance to disseminating prostate cancer cells

Sara Roccabianca[1#], Elisa Pardella[1#], Luigi Ippolito[1], Erica Pranzini[1], Marta Iozzo[1], Giulia Gangarossa[1], Greta Bertoli[1], Giacomo Venditti[1], Caterina Grillo[1], Giuseppina Comito[1], Paola Chiarugi[1], Elisa Giannoni[1]

[1]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, 50134 Florence, Italy; sara.roccabianca96@gmail.com

[#]These authors contributed equally to this work.

Background: Metastasis is a catastrophic yet rare phenomenon, due to several stresses that disseminating tumor cells (DTCs) are exposed to during its multiple stages, such as the elevated ROS levels in the bloodstream. The exposure to such oxidative stress makes DTCs more susceptible to ferroptosis, an iron-dependent form of regulated cell death triggered by the iron-dependent lethal accumulation of ROS and extensive membrane lipid peroxidation.

Aim: DCCs that acquire resistance to ferroptosis are more likely to successfully metastasize, thus we aim to characterize this phenomenon to identify actionable vulnerabilities against prostate cancer (PCa) progression. To do so, we compared cells growing in adherence (2D model) to cells cultured in suspension conditions (3D model).

Results: Despite exhibiting increased ROS levels compared to 2D cultures, 3D models showed reduced membrane lipid peroxidation, indicating a decreased susceptibility to oxidative damage independent of the canonical xCT/GSH/GPX4 axis, but rather relying on alternative mechanisms. Notably, our study revealed that the GOT2-dependent synthesis of aspartate fuels the Dihydroorotate Dehydrogenase (DHODH)-driven regeneration of ubiquinol at the mitochondrial membrane, acting as an alternative radical-trapping antiferroptotic strategy. Genetic targeting of DHODH and GOT2 *in vitro* led to a loss of the ferroptosis-resistant phenotype rescued by the treatment with exogenous ubiquinol, corroborating our previous findings. Strikingly, *vivo* studies showed that downregulation of DHODH significantly impaired the survival of DCCs, confirming its relevance in enabling PCa dissemination. Finally, treatment with DHODH inhibitors proved to be an efficient strategy to induce cell death in 3D cultures.

Conclusions: Our data provide novel insights on the mechanisms underlying ferroptosis resistance during PCa metastatic dissemination, opening new perspectives on ferroptosis targeting as a potential therapeutic strategy.

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Poster 62

RAC signaling links melanoma cell plasticity to ferroptosis susceptibility

Patrizia Romani[1]

[1]Dipartimento di Medicina Molecolare, Università di Padova, patrizia.romani@unipd.it

Background: Melanoma exhibits remarkable phenotypic plasticity, allowing tumor cells to switch between distinct invasive states and adapt to environmental stress. Increasing evidence suggests that these transitions are associated with metabolic rewiring and altered susceptibility to ferroptosis, an iron-dependent form of regulated cell death. RAC GTPases are key regulators of melanoma cell behavior, but their role in coordinating these processes remains poorly understood.

Aim: To investigate the contribution of RAC signaling to melanoma cell plasticity and to determine its impact on metabolic homeostasis and ferroptosis susceptibility.

Results: Our findings indicate that modulation of RAC activity influences the acquisition of invasive melanoma phenotypes and is associated with changes in cellular pathways involved in redox balance, mitochondrial function and ferroptosis regulation. These observations suggest a functional link between RAC-dependent plasticity programs and metabolic vulnerabilities in melanoma cells.

Conclusions: The identification of RAC-dependent mechanisms connecting invasive behavior and ferroptosis sensitivity provides new insights into melanoma biology and may reveal novel therapeutic opportunities to target aggressive melanoma cell populations.

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Poster 63

An engineered cell culture device for reproducing tumor oxygen conditions *in vitro*

Mariella Rosalia[1,2], Marco Malferrari [2], Enrico Grassilli [1] and Stefania Rapino [2]

[1]InSimili srl, Bologna, Italy; mrosalia@insimili.com; egrassilli@insimili.com
[2]Department of Chemistry "Giacomo Ciamician", University of Bologna, Bologna, Italy; marco.malferrari2@unibo.it; stefania.rapino3@unibo.it.

Background: Hypoxia is a hallmark of cancer, as 90% of solid tumors thrive in hypoxic microenvironments. Cellular responses to low oxygen are mediated by hypoxia-inducible factors (HIFs), controlling metabolic and transcriptional adaptation and favoring cancer cell proliferation, migration and invasion. However, in vitro anticancer drug screenings are often performed under standard conditions (~19% O_2), due to the technical challenges and high costs associated with hypoxia workstations and incubators. These hyperoxic conditions alter gene expression and metabolic behavior, confounding the relevance of results and delaying the development of new therapies.

Aim: This work showcases an innovative multiwell cell culture device (OxygenControl plate) that exploits a functional biomaterial to regulate oxygen concentration in vitro, without the need for hypoxia workstations, chambers, or special incubators.

Results: The OxygenControl plate successfully controlled oxygen concentration in the cell culture medium, reaching target levels (1%, 3%, 5% O_2) within minutes. Oxygen levels and their spatial distribution were monitored by scanning electrochemical microscopy (SECM) using a 10 µm platinum microelectrode, confirming homogeneous concentrations across all tested well formats (6-, 12-, 24-, and 96-well plates) and stability for up to 5 days. Biological validation with cancer cell lines confirmed full device compatibility, with cell growth curves showing normal proliferation. Fluorescence microscopy and Western blotting further demonstrated HIF stabilization.

Conclusions: The OxygenControl plate is an effective, user-friendly tool for controlling oxygen levels in vitro, providing a pathophysiologically relevant microenvironment for cancer research and drug screening applications.

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Poster 64

Osteogenic differentiation reshapes mitochondrial organization and metabolism in osteosarcoma cells

Francesca Rossi[1], Lorenzo Anconelli[1], Martina Rossi[1], Concettina Cappadone[1], Stefano Iotti[1], Giulia Vignoli[1], Anna Maria Ghelli[1], Manuela Sollazzo[1], Gloria Ravegnini[1], Francesca Gorini[1] and Emil Malucelli[1]

[1]Department of Pharmacy and Biotechnology, University of Bologna, 33-40126 Bologna, Italy

francesca.rossi105@unibo.it, lorenzo.anconelli@unibo.it, martina.rossi12@unibo.it, concettina.cappadone@unibo.it, stefano.iotti@unibo.it, giulia.vignoli8@studio.unibo.it, annamaria.ghelli@unibo.it, manuela.sollazzo2@unibo.it, gloria.ravegnini2@unibo.it, francesca.gorini3@unibo.it, emil.malucelli@unibo.it

Background: Osteosarcoma (OS) is the most common primary malignant bone tumor and is characterized by impaired osteogenic differentiation and defective biomineralization. Increasing evidence suggests that mitochondria play a key role in calcium handling and mineral formation during osteogenesis [1,2]. However, the relationship between mitochondrial remodeling, metabolism, and osteogenic differentiation remains unclear.

Aim: This study investigated mitochondrial dynamics and metabolic reprogramming during osteogenic differentiation of SaOS-2 cells.

Results: Synchrotron X-ray tomography revealed significant mitochondrial remodeling during osteogenic induction in 2D cultures. Non- induced cells displayed elongated mitochondria, whereas induced cells showed spherical mitochondria at early stages (4 days), consistent with mitochondrial fission. At later stages (10 days), mitochondria appeared fused and interconnected through nanotunnel-like structures. These structural changes were accompanied by metabolic reprogramming. Seahorse analysis demonstrated increased maximal respiration and ATP-linked oxygen consumption in induced cells at 4 days, suggesting enhanced energetic demand during differentiation. Resipher measurements confirmed higher cellular respiration in induced cultures at early time points, while non-induced cells exhibited greater oxygen consumption at later stages, suggesting differential metabolic adaptation over time. Osteogenic differentiation was confirmed by PCR showing overexpression of COL1, BGLAP, and SPP1 in 10 days. In parallel, 3D spheroid cultures made of OS and human bone marrow mesenchymal stem cells were established and maintained viable over time.

Conclusions: These findings highlight a coordinated mitochondrial and metabolic reprogramming in OS differentiation, underpinning a differentiation-driven metabolic shift and providing a foundation for novel therapeutic strategies.

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References

1 ROSSI, Francesca, et al. Shedding light on osteosarcoma cell differentiation: impact on biomineralization and mitochondria morphology. International Journal of Molecular Sciences, 2023, 24.10: 8559.

2 BOONRUNGSIMAN, Suwimon, et al. The role of intracellular calcium phosphate in osteoblast-mediated bone apatite formation. Proceedings of the National Academy of Sciences, 2012, 109.35: 14170-14175

Poster 65

Spatiotemporal cellular responses to Trastuzumab-Deruxtecan; lysosomal hubs and organelle triads in HER2+ breast cancer cells

Shahnaz Salamat[1], Erica Tagliatti[2], Maria Cristina Gagliani[1], Grazia Bellese[1], Michele Sorrenti[1], Martina Crippa[3], Manuela Sollazzo[4], Andrea Abbona[5,6], Matteo Paccagnella[5,6], Pietro Arnaldi[1,11], Marco Carlo Merlano[5,7], Ornella Garrone[8], Annamaria Porcelli[4], Michela Matteoli[2], Paola Falletta[3,9], Patrizio Castagnola[10], Katia Cortese[1,10]

[1]*DIMES, Department of Experimental Medicine, Cellular Electron Microscopy Lab, University of Genoa, Genoa, Italy.*
[2]*IRCCS Humanitas Research Hospital, Laboratory of Pharmacology and Brain Pathology, Rozzano, Milano, Italy.*
[3]*Experimental Imaging Center, IRCCS Ospedale San Raffaele, Milan, Italy.*
[4]*FABIT, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy.*
[5]*Fondazione Arco Cuneo, Italy.*
[6]*Health Department S. Croce e Carle Teaching Hospital, Cuneo, Italy*
[7]*Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Italy.*
[8]*Medical Oncology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.*
[9]*Vita-Salute San Raffaele University, Milan, Italy.*
[10]*IRCCS Ospedale Policlinico San Martino, Genova, Italy.*

Affiliation 1; shahnaz.salamat@edu.unige.it
Affiliation 2; cortesek@unige.it

Background: Trastuzumab–deruxtecan (T-DXd) demonstrated strong clinical efficacy in HER2$^+$ and HER2-low breast cancers, however, its intracellular mechanisms underlying its action remain poorly understood.

Aim: To investigate T-DXd dynamics in HER2$^+$ breast cancer cells, we implemented multimodal imaging techniques combining live-cell imaging, confocal microscopy, immuno-EM, and biochemical assays. HER2 over-expressing SK-BR-3 cells were exposed to T-DXd (10 µg/mL for 2–72 h) and compared to IgG controls.

Results: During the adaptation phase (2–24 h), we observed HER2 phosphorylation, sustained ERK activation despite a decrease in total ERK, and down-regulation of AKT signaling. Simultaneously, TFEB and LAMP1 expression increased, suggesting early lysosomal biogenesis. At 24 h, we observed the unexpected release of T-DXd–positive sphereosomes, a specialized form of extracellular vesicles revealed by under electron microscopy. At later stages (48–72 h), live-cell imaging showed maximal intracellular accumulation of T-DXd–HER2, while confocal and immuno-EM imaging revealed that these assemblies concentrated within enlarged, metabolically active lysosomes that formed tripartite contact sites with both mitochondria and the nucleus. The formation of these structures coincided with γH2AX activation, Lamin B1 upregulation, and marked nuclear envelope stress.

Conclusions: Collectively, these findings illustrate how imaging uncovers ADC-induced cellular remodeling and pinpoint lysosomal hubs, and ERK signaling as possible targets for combination therapy, highlighting the value of advanced multimodal workflows in defining ADC mechanisms of action.

Poster 66

5-Aminomethyl-8-hydroxyquinoline Triggers Cuproptosis in Colon Cancer Cells by Acting as a Copper Ionophore

Alessandra Folda[1], Alessia Distefano[2], Alessia Sambugaro[3], Erik Murador[3], Maria Pia Rigobello[1], Chiara Nardon[3], Valentina Oliveri[2] and **Valeria Scalcon**[1]

[1]Dept. Biomedical Sciences, University of Padova, Padova, Italy
[2]Dept. Chemical Sciences, University of Catania, Catania, Italy
[3]Dept. Biotechnology, University of Verona, Verona, Italy

email: valeria.scalcon@unipd.it

Background: Copper (Cu) homeostasis dysregulation has emerged as a key feature of cancer cells, offering a potential vulnerability to exploit therapeutically. Among the strategies to target copper metabolism, ionophore-based approaches have gained increasing attention for their ability to selectively promote copper-dependent cell death, known as cuproptosis. However, the search for quinoline derivatives endowed with cuproptosis-inducing properties remains largely unexplored, representing a compelling avenue for the development of novel and selective anticancer agents.

Aim: We investigated various quinolines in combination with Cu^{2+} against colon cancer cell models and focused on the most active 5-aminomethyl-8-hydroxyquinoline (5-AMHQ) exploring its anticancer potential and mechanism of action.

Results: 5-AMHQ/Cu^{2+} showed significant cytotoxicity in colon cancer cells. Mechanistic studies revealed redox imbalance, evidenced by decreased cellular thiols and increased glutathione oxidation. Furthermore, 5-AMHQ/Cu^{2+} altered mitochondrial oxygen consumption and elevated ROS levels, confirming oxidative stress induction. Transmission electron microscopy revealed significant alterations in mitochondrial morphology in treated cells. Further investigation confirmed DLAT oligomerization, a hallmark of cuproptosis, supporting a copper-dependent mechanism of cell death.

Conclusions: 5-AMHQ acts as a copper ionophore triggering cuproptosis in colon cancer cells, as evidenced by DLAT oligomerization and mitochondrial dysfunction. These findings highlight its promising anticancer potential and encourage further investigation toward more selective and effective treatments.

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Poster 67

Lactate fuels acetyl-CoA dependent epigenetic remodeling in germinal center B cells

Nilde Scalisi[1], Beatrice Calciolari[2], Marco Fantuz[3], Marta Iozzo[4], Mattia Albiero[5], Roberta Noè[6], Katia Basso[7], Sophie Trefely[8], Paola Chiarugi[9], Simone Sidoli[10], Alessandro Carrer[11]

[1]Department of Biology, University of Padova, Padua, Italy/ Veneto Institute of Molecular Medicine (VIMM), Padua, Italy. nilde.scalisi@studenti.unipd.it / nilde.scalisi@vimm.it
[2]Veneto Institute of Molecular Medicine (VIMM), Padua, Italy. beatrice.calciolari@vimm.it
[3]Veneto Institute of Molecular Medicine (VIMM), Padua, Italy. marco.fantuz@vimm.it
[4]Department Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Firenze, Italy. marta.iozzo@unifi.it
[5]Department Department of Surgery, Oncology and Gastroenterology, University of Padova, Padua, Italy. mattia.albiero@unipd.it
[6]Department of Biology, University of Padova, Padua, Italy/ Veneto Institute of Molecular Medicine (VIMM), Padua, Italy roberta.noe@studenti.unipd.it / roberta.noe@vimm.it
[7]Institute for Cancer Genetics, Columbia University, New York, NY, USA. kb451@columbia.edu
[8]Epigenetics and Signalling Programs, Babraham Institute, Cambridge, UK. sophie.trefely@babraham.ac.uk
[9]Department Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Firenze, Italy paola.chiarugi@unifi.it
[10]Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY, USA simone.sidoli@einsteinmed.edu
[11]Department of Biology, University of Padova, Padua, Italy/ Veneto Institute of Molecular Medicine (VIMM), Padua, Italy. alessandro.carrer@unipd.it / alessandro.carrer@vimm.it

Background: Epigenetic and metabolic rewiring are pivotal in B lymphocyte maturation and differentiation during the germinal center (GC) reaction. Although GC B cells undergo profound metabolic and chromatin remodeling upon activation, it remains unclear whether metabolic changes directly drive epigenetic rewiring.

Aim: We aimed to define the metabolic mechanisms that couple metabolism to chromatin remodeling in GC B cells. Specifically, we investigated whether acetyl-CoA metabolism and lactate utilization regulate histone acetylation and B cell proliferation in pathophysiological contexts such as Diffuse Large B Cell Lymphoma (DLBCL) and aging.

Results: GC-mimicking stimulation of murine and human B cells induced profound metabolic rewiring, with increased TCA cycle activity and acetyl-CoA **Results**: GC-mimicking stimulation of murine and human B cells induced profound metabolic rewiring, with increased TCA cycle activity and acetyl-CoA production. We identified CD40 signaling as the major upstream driver of AKT-dependent ATP citrate lyase (ACLY) activation, promoting histone hyperacetylation and cell cycle progression. Using isotope tracing approaches, we identified lactate as a major carbon source fueling acetyl-CoA synthesis and H3K27 acetylation (H3K27-ac). Genome-wide epigenomic profiling (CUT&Tag) further revealed lactate-dependent chromatin remodeling programs linked to Cbl signaling-associated loci. Importantly, GC-derived DLBCL cells hijacked this metabolic-epigenetic axis, displaying elevated ACLY activation and sensitivity to ACLY and BET inhibition. Finally, aging-associated defects in GC histone acetylation reflected reduced lactate uptake within the GC niche, rather than impaired ACLY activation.

Conclusions: Our findings reveal a GC-associated metabolic-epigenetic axis linking lactate metabolism to histone acetylation in GC B cells, suggesting metabolic interplay between B and T cells with implications for immunity and lymphomagenesis.

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Poster 68

Fenretinide-loaded human serum albumin nanoparticles impair tumor metabolism and induce apoptosis in 3D neuroblastoma spheroids

Luca Schiassi[1], Lorenzo Anconelli[1], Martina Rossi[1], Francesca Farioli[1], Francesca Gorini[1], Gloria Ravegnini[1], Francesca Rossi[1], Davide Piras[2], Andrea Cristaldi[2], Concettina Cappadone[1], Cecilia Garofalo[3], Giovanna Farruggia[1], Isabella Orienti[1], Emil Malucelli[1] and Stefano Iotti[1]

[1]Department of Pharmacy and Biotechnology, Alma Mater Studiorum University of Bologna, Bologna, Italy.
[2]CellDynamics iSRL, Bologna, Italy
[3]IRCCS Istituto delle Scienze Neurologiche di Bologna, Via Altura 3, 40139 Bologna, Italy.

Background: Therapeutic regimens for pediatric malignancies such as neuroblastoma remain highly intensive and are often associated with severe systemic toxicity. Fenretinide (FEN), a synthetic retinoid derivative, has shown promising antitumor activity; however, its clinical use is hampered by poor aqueous solubility and low bioavailability. Nanotechnology represents a promising strategy to improve drug solubility, delivery, and therapeutic efficacy.

Aim: This project aims to develop FEN-loaded human serum albumin nanoparticles (HSA-F) to enhance FEN solubility and bioavailability, and to evaluate their therapeutic efficacy in a 3D SH-SY5Y neuroblastoma spheroid model.

Results: The HSA-F formulation showed optimal physicochemical properties i.e.:
i)a monodisperse size distribution of 100-200 nm, ii) negative zeta potential, and
ii)improved aqueous solubility. In 3D SH-SY5Y spheroids, HSA-F induced significantly higher cytotoxicity than free FEN and altered spheroid physical parameters. Analysis of cell death mechanisms revealed mitochondrial dysfunction and marked activation of caspase-9 and caspase-3/7, confirming apoptosis induction. Gene expression analysis showed modulation of tumor survival and metabolism-associated pathways, with downregulation of c-MYC and reduced expression of the lipid metabolism-related enzyme ACAT2. In parallel, HSA-F upregulated TNF-α, suggesting activation of the extrinsic apoptotic pathway, and modulated the endocytic regulator SGIP1.

Conclusions: HSA-F nanoparticles overcome the poor water solubility of FEN improving the bioavailability and enhancing its antitumor activity in 3D neuroblastoma spheroids. Their efficacy is associated with improved spheroid penetration, mitochondrial-mediated apoptosis, and modulation of genes involved in tumor survival, lipid metabolism, apoptosis, and endocytosis. These findings support HSA-F as a promising nanomedicine platform for targeted therapy in high-risk neuroblastoma.

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Poster 69

Paraoxonase-2 immunoexpression correlates with tumor grade and aggressiveness in clear cell renal cell carcinoma

Valentina Schiavoni[1], Roberta Mazzucchelli[2], Monia Cecati[3], Valentina Pozzi[1], Gaia Goteri[2], Roberto Campagna[3], Eleonora Salvolini[1] and Davide Sartini[1]

[1]Department of Clinical Sciences, Polytechnic University of Marche, Ancona, Italy. v.schiavoni@pm.univpm.it; v.pozzi@univpm.it; e.salvolini@univpm.it; d.sartini@univpm.it
[2]Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy. r.mazzucchelli@univpm.it; g.goteri@univpm.it
[3]Department for the Promotion of Human Science and Quality of Life, San Raffaele Roma University, Rome, Italy. monia.cecati@uniroma5.it; roberto.campagna@uniroma5.it

Background: Paraoxonase-2 (PON2) is an intracellular antioxidant enzyme involved in redox homeostasis and the regulation of cell survival pathways. Given the growing evidence implicating PON2 in cancer biology and tumor progression, its potential role may be particularly relevant in aggressive malignancies such as clear cell renal cell carcinoma (ccRCC). ccRCC remains associated with high mortality despite advances in diagnosis and treatment, largely because of its aggressive behavior and frequent late-stage detection. However, the expression profile and clinicopathological significance of PON2 in ccRCC remain unexplored.

Aim: This study aimed to investigate the immunohistochemical expression of PON2 in ccRCC tissues and to evaluate its association with clinicopathological parameters, including tumor grade, stage, and patient sex.

Results: A total of 79 formalin-fixed paraffin-embedded ccRCC samples were retrospectively analyzed. PON2 expression was assessed by immunohistochemistry using a semi-quantitative staining score based on staining intensity and percentage of positive cells. Statistical analysis demonstrated no significant association between PON2 expression and patient sex or tumor stage. Conversely, enzyme immunoexpression was found to be significantly correlated with tumor grade. Mean staining scores progressively increased according to tumor grade, from 104.76 ± 23.86 in grade 1 tumors to 214.72 ± 14.17 in grade 4 tumors. High-grade tumors showed markedly stronger PON2 expression compared with low-grade lesions, suggesting a relationship between PON2 overexpression and tumor aggressiveness.

Conclusions: PON2 expression is significantly associated with increasing tumor grade in ccRCC, supporting its potential involvement in tumor progression and aggressiveness. These results suggest that PON2 may represent a promising prognostic biomarker and a possible therapeutic target in ccRCC.

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Poster 70

Transglutaminase Type 2 regulates mtDNA transcription, chemoresistance and immune cell recruitment in melanoma

Filippo Severin[1], Federica Vianello[1], Sara Coletta[1], Alessia Ruzza[1], Elisabetta Zaltron[1], Claudio Laquatra[2], Angelo Velle[1], Ilaria Celotti[1], Matteo Scavezzon[1], Sabrina Toffanin[1], Aurora Madiolo[1], Lara Iocco[1], Annalisa Paronetto[1], Michela Rossini[1], Andrea Rasola[2], Chiara Romualdi[1], Marina De Bernard[1], Luigi Leanza[1]

[1]Department of Biology, University of Padova; filippo.severin@unipd.it; luigi.leanza@unipd.it
[2]Department of Biomedical Sciences, University of Padova

Background: Transglutaminase 2 (TG2) is a multifunctional protein crucially involved in cell survival and drug resistance. In cutaneous melanoma, TG2 acts as a positive prognostic factor, as its downregulation correlates with invasive phenotypes and increased metastatic potential. Recent in silico and KO models suggest a pivotal role for TG2 in modulating the tumor microenvironment (TME) by influencing immune cell recruitment and activation (e.g., CD4+ T-cells). However, the specific link between TG2 and immunometabolism remains poorly understood. Elucidating how TG2 coordinates metabolic programs and immune responses is essential for overcoming immune escape and developing novel therapeutic strategies involving dietary and metabolic modulation.

Aim: This study aims to investigate the role of Transglutaminase Type 2 (TG2) in modulating the immune microenvironment and mitochondrial metabolism in skin cutaneous melanoma, specifically evaluating how TG2 influences immune cell recruitment and mitochondrial respiratory chain activity to mediate chemosensitivity.

Results: TG2 KO cells displayed reduced expression and activity of mitochondrial respiratory chain complexes due to TG2-dependent mtDNA transcription modulation, leading to increased susceptibility of TG2-deficient cells to chemotherapeutic agents. In addition, co-culture analyses suggest that TG2 loss affects immune cell dynamics, particularly on the recruitment of both monocytes and lymphocytes, pointing to a regulatory role of TG2 in immune microenvironment shaping.

Conclusions: TG2 emerges as a key regulator of mitochondrial metabolism and immune modulation in melanoma. Its targeting may enhance therapeutic responses or help overcome drug resistance.

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Poster 71

Analysis of circulating extracellular vesicles as a non-invasive biomarker in pancreatic cancer

Giada Silvi[1], Maria Francesca Paolocci[1], Lorenzo Depau[1] Lapo Bencini[2], Chiara Falciani[1], Luisa Bracci[1] and Jlenia Brunetti[1]

[1]Department of Medical Biotechnologies, University of Siena, jlenia.brunetti@unisi.it;
[2]Department of Oncology and Robotic Surgery, Careggi Main Florence University and Regional Hospital, bencinil@aou-careggi.toscana.it

Background: Pancreatic cancer is one of the most lethal malignancies due to frequent late diagnosis and the lack of early, specific biomarkers. In this context, liquid biopsy based on the analysis of extracellular vesicles (EVs) emerges as a promising tool for non-invasive screening and disease monitoring. EVs, which are secreted in higher quantities by tumor cells, carry bioactive molecules that reflect the state of their cell of origin, offering a stable source of circulating biomarkers.

Aim: The aim of this study was to characterize the surface marker profile of plasma-derived EVs from pancreatic cancer patients and evaluate their potential role as circulating biomarkers.

Results: EVs were isolated using a commercially available kit from plasma samples of pancreatic cancer patients and healthy volunteers and were characterized by nanoparticle tracking analysis and multiplex bead-based flow cytometry to detect 37 exosomal surface epitopes. Comparative analysis revealed significantly increased expression of HLA-ABC, CD3, and CD45 ($p < 0.05$) in EVs derived from pancreatic cancer patients, suggesting a strong contribution of immune-derived vesicles to the circulating pool. Conversely, markers such as CD56, CD44, and CD24 were significantly down-regulated in tumor-derived EVs compared with healthy controls.

Conclusions: The results reveal a distinctive protein signature in the circulating EVs of pancreatic cancer patients with respect to healthy volunteers. The increase in immune-related markers, as HLA-ABC, CD3 and CD45, and the reduction of CD56, CD44 and CD24 may reflect alterations in the tumor microenvironment and systemic anti-tumor immunity. This combined approach opens new perspectives for the development of more accurate diagnostic and prognostic tests for pancreatic cancer.

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UNTANGLING THE MOLECULAR AND METABOLIC NETWORKS IN CANCER PROGRESSION

6th Workshop of the SIB group "Tumor Biochemistry"
Bologna, July 9-10th, 2026

Poster 72

CYP1A1 regulates estrogen receptor activity and response to CDK4/6 inhibitors in estrogen receptor-positive breast cancer cells

Gabriele Tani[1], Alfredo Smiriglia[1], Marina Bacci[1], Nicla Lorito[1], Francesca Bonechi[1], Alice Santi[1], Gonzàlez-Fernàndez Martìn[2,3], Sven Rottenberg[2,3], Paola Francica[2,3], Andrea Morandi[1,2]

[1]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy;
[2]Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland;
[3]Cancer Therapy Resistance Cluster, Department for BioMedical Research, University of Bern, Bern, Switzerland

Background: Estrogen receptor positive (ER+) tumors are the most common breast cancer molecular subtype. Endocrine therapy (ET) combied with CDK4/6 inhibitors (CDK4/6i) is the standard-of-care (SOC) therapy for advanced and high-risk ER+ breast cancer, but resistance may limit its efficacy. Metabolic reprogramming drives therapeutic adaptation, highlighting the possibility to identify novel metabolic vulnerabilities.

Aim: This project elucidates the role of cytochrome P450-1A1 (CYP1A1), an enzyme involved in the generation of oxysterols and epoxides, which emerged from a *loss-of-function* metabolic screen as a sensitizer to CDK4/6i in a breast cancer model of ET resistance (MCF7-LTED cells).

Results: CYP1A1 knock-down synergizes with CDK4/6 inhibitors and induces a ROS-dependent cell death, an effect that is rescued by the antioxidant N-acetylcysteine (NAC). This effect was associated with reduced ER transcriptional activity and phosphorylation, supporting a key role for CYP1A1 in sustaining ER signaling in MCF7-LTED cells. Mechanistically, CYP1A1 produces cholesterol-derived metabolites, specifically 5,6-cholesterol epoxides (EC) and 6-oxo-cholestane-3beta,5alpha-diol (OCDO). Exogenous administration of EC and/or OCDO induced ER activation, as shown by immunofluorescence of phosphorylated ER at Ser118 and the activation of ER-target genes, thereby abrogating the synthetic lethality induced by CYP1A1 silencing. We further identified that intracellular cholesterol is mobilized from lipid droplets through carboxylesterase 1 (CES1)-mediated hydrolysis of cholesteryl esters; accordingly, CES1 inhibition synergized with CDK4/6i to induce synthetic lethality, an effect that was reversed by exogenous cholesterol.

Conclusions: Our findings uncover a metabolically driven mechanism underlying CDK4/6i response and identify CYP1A1 as a potential predictive biomarker and therapeutic target in ER+ breast cancer.

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Poster 73

Taming the metabolism of tumor associated macrophages to fight peripheral nerve neoplasms

Margherita Toffanin[1,2], Maria Egle Castorina[1,2], Francesca Scantamburlo[1], Marco Fantuz[3], Alessandro Carrer[3,4] Andrea Rasola[1] and Ionica Masgras[2]

[1]Department of Biomedical Sciences, University of Padova, Padova, Italy; margherita.toffanin@studenti.unipd.it; mariaegle.castorina@studenti.unipd.it; francesca.scantamburlo@studenti.unipd.it; andrea.rasola@unipd.it
[2]Neuroscience Institute, National Research Council, Padova, Italy; ionica.masgras@cnr.it
[3]Veneto Institute of Molecular Medicine, Padova, Italy; marco.fantuz@unipd.it
[4]Department of Biology, University of Padova, Padova, Italy; alessandro.carrer@unipd.it

Background: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft-tissue sarcomas of peripheral nerves, representing a major cause of mortality in patients with Neurofibromatosis type 1 (NF1). NF1 is an autosomal dominant disorder caused by germline inactivating mutations in the neurofibromin gene, a negative regulator of the oncogene p21 RAS. Loss of neurofibromin leads to the development of multiple types of benign tumors, such as plexiform neurofibromas (PNFs) from which MPNSTs arise following the accumulation of additional genetic alterations in Schwann cells. Beyond tumor cell–intrinsic alterations, the tumor microenvironment plays a crucial role in disease progression. Tumor-associated macrophages (TAMs), mainly characterized by an M2-like phenotype, constitute the most abundant immune population and promote tumor growth by enhancing angiogenesis, invasion, and immune evasion.

Aim: For this reason, the aim of this project is to investigate the crosstalk between MPNST cells and macrophages, in order to identify macrophage-specific metabolic actors sustaining tumor growth.

Results: Transcriptomic profiling by RNA sequencing of macrophages exposed to conditioned medium derived from an MPNST cell line revealed alterations in the expression of genes associated with cholesterol metabolism. Our objective is therefore to determine whether dysregulation of cholesterol-related pathways contributes to the polarization of macrophages toward a pro-tumoral phenotype. Notably, this macrophage transcriptional response is lost when the mitochondrial chaperone TRAP1 is knocked out. TRAP1 deficiency also prevents the induction of immunosuppressive gene expression in conditioned macrophages, indicating its key role in mediating tumor-induced macrophage reprogramming.

Conclusions: These findings highlight TRAP1 as a regulator of macrophage polarization in the tumor microenvironment and warrant further investigation to identify the underlying molecular mechanisms. Therefore, we aim to identify metabolic vulnerabilities in the macrophage population that may represent promising candidates for the development of novel therapeutic strategies.

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Poster 74

Spermidine and Eugenol Modulate Tight Junction and Stemness Markers in Colorectal Cancer Spheroids

Marcella Turzi[1], Silvia Dilloo[2], Giovanni Dinelli[3] and Francesca Truzzi[4]

[1]marcella.turzi2@unibo.it
[2]silvia.dilloo2@unibo.it
[3]giovanni.dinelli@unibo.it
[4]francesca.truzzi3@unibo.it

Background: Colorectal cancer (CRC) progression is associated with alterations in tight junction (TJ) proteins and dysregulation of cancer stem cell (CSC)-related markers, which contribute to tumor aggressiveness, metastasis and therapy resistance. Natural bioactive compounds such as spermidine (SPD) and eugenol (EUG) have shown potential anti-cancer properties, although their combined effects on CSC- and TJ-associated pathways in CRC spheroids remain poorly understood.

Aim: This study aimed to evaluate the effects of SPD and EUG, administered alone or in combination, on spheroid growth, self-renewal capacity and the expression of TJ- and CSC-associated markers in Caco-2- and SW620-derived colorectal cancer spheroids, representative of primary and metastatic CRC models.

Results: Treatment with SPD+EUG significantly reduced spheroid area and self-renewal capacity in both Caco-2 and SW620 spheroids, with stronger effects observed when treatment was administered at the time of seeding. Baseline profiling showed higher TJ marker expression in Caco-2 spheroids, whereas metastatic SW620 spheroids exhibited increased expression of stemness-related markers. SPD and EUG modulated the expression of CSC- and TJ-related genes, including Occludin, Zonulin, CD133, ALDH1A1, SOX2 and VE-cadherin. However, transcript changes were not consistently reflected at the protein level, suggesting the involvement of post-transcriptional regulatory mechanisms.

Conclusions: SPD and EUG, particularly in combination, reduced spheroid growth and self-renewal capacity in both primary and metastatic CRC models. Treatment-induced modulation of TJ- and CSC-associated markers was observed, although transcript and protein expression profiles were not always consistent, suggesting post-transcriptional regulation.

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Poster 75

Deregulation of mitochondrial metabolism and NAD homeostasis during prostate cancer cell dissemination

Giacomo Venditti[1], Luigi Ippolito[1], Erica Pranzini[1], Sara Roccabianca[1], Marta Iozzo[1], Greta Bertoli[1], Caterina Grillo[1], Giuseppina Comito[1], Elisa Giannoni[1] and Paola Chiarugi[1]

[1]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence (Italy); giacomo.venditti@unifi.it

Background: Metastatic dissemination is the leading cause of mortality in prostate cancer (PC). To successfully metastasize, cancer cells must overcome multiple stresses, including extracellular matrix detachment and *anoikis*, circulation in the bloodstream, and colonization of distant organs, all requiring substantial metabolic adaptations.

Aim: This study aims to identify metabolic vulnerabilities of metastasizing PC cells that could be therapeutically exploited to reduce metastatic burden.

Results: We established a comparative *in vitro* model using PC cells cultured either as 2D monolayers (2D-C), representing the anchorage-dependent cells in the primary tumor, or as non-adherent 3D cultures (3D-C), mimicking the loss of stromal support encountered by metastatizing cells. Compared with 2D-C, 3D-C displayed increased mitochondrial mass and elevated expression of electron transport chain (ETC) complexes. However, mitochondria from 3D-C were functionally compromised, showing reduced oxidative phosphorylation and impaired ETC complex I and II activity. Mitochondrial dysfunction in 3D-C was associated with altered redox homeostasis, characterized by an increased NADH/NAD^+ ratio indicative of reductive stress. Since NAD^+ is an essential cofactor for metabolic dehydrogenases and a substrate for enzymes involved in DNA repair and stress responses, maintaining NAD^+ availability appears critical for survival during dissemination. Detached PC cells upregulated nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of the NAD^+ salvage pathway. We hypothesize that NAMPT sustains NAD^+ regeneration under reductive stress, promoting survival of disseminating cells and representing a therapeutic vulnerability.

Conclusions: Detached PC cells exhibit mitochondrial dysfunction and redox imbalance, resulting in increased dependence on the NAD^+ salvage pathway. Targeting NAMPT-mediated NAD^+ regeneration may represent a promising strategy to limit metastatic dissemination.

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Poster 76

Affibody molecules as candidates for multiple myeloma treatment

Cristina Visentin[1], Laure Martine Gauthier-Manuel[2], Nour Alhoda Kassab[3], Giulia Rizzi[4], Riccardo Capelli[5], Stefano Ricagno[6]

[1]Dipartimento di Bioscienze – Università degli Studi di Milano; cristina.visentin@unimi.it
[2]Dipartimento di Bioscienze – Università degli Studi di Milano; laure.gauthier-manuel@unimi.it
[3]Dipartimento di Bioscienze – Università degli Studi di Milano; nouralhoda.kassab@studenti.unimi.it
[4]Dipartimento di Bioscienze – Università degli Studi di Milano; giulia.rizzi2@unimiu.it
[5]Dipartimento di Bioscienze – Università degli Studi di Milano; riccardo.capelli@unimi.it
[6]Dipartimento di Bioscienze – Università degli Studi di Milano; stefano.ricagno@unimi.it

Background: Multiple myeloma is a treatable but currently incurable disease characterized by the abnormal proliferation of plasma cells. Several biomarkers are used to monitor disease progression and define clinical stages, among which β2microglobulin (β2m) is one of the most reliable. β2m progressively accumulates and contributes to chronic inflammation. It aggregates within lysosomes of tumor-associated macrophages, causing lysosomal rupture and release of their content. This process promotes the secretion of pro-inflammatory cytokines, ultimately supporting B-cell proliferation.

Aim: To identify molecules capable of interfering with pathological β2m aggregation under lysosomal-like conditions.

Results: Affibody molecules were expressed and purified. Their stability at acidic pH was first assessed, confirming their resilience under lysosomal-like conditions. Binding to β2m at pH 4.5 was demonstrated
for Zβ2m_01 and Zβ2m_02 using size exclusion chromatography, microscale thermophoresis, and isothermal titration calorimetry. Aggregation assays further showed that Zβ2m_01 effectively inhibits β2m aggregation at acidic pH.

Conclusions: Affibody molecules represent promising candidates for multiple myeloma treatment, given their ability to bind β2mand retain stability under harsh environmental conditions.

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Poster 77

The effect of the mevalonate pathway on the PD-L1/PD-L2 signaling axis in BRAF^{mut} against NRAS^{mut} melanoma

Annalena Weber[1], Raul Castro-Portuguez[1], Stefania Vassallo[2], Alfredo Pherez Farah[3], Pietro Lugato[1], Roxana E. Oberkersch[1] and Massimo M. Santoro[1]

[1]Laboratory for Angiogenesis and Cancer Metabolism, Department of Biology, University of Padova; annalena.weber@studenti.unipd.it; joseraul.castroportuguez@unipd.it; roxanaelena.oberkersch@unipd.it; massimo.santoroy@unipd.it
[2]Department of Biology, University of Padova; stefania.vassallo.2@phd.unipd.it
[3]Laboratory of Synthetic Immunology, Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy.; alfredo.pherezfarah@studenti.unipd.it

Background: Melanoma is the most aggressive form of skin cancer and is driven by hyperactivation of the MAPK signalling pathway. Approximately 50% of patients harbour BRAF mutations (BRAF V600E), while almost 20% carry NRAS mutations (NRAS Q61R). Although targeted therapies, like specific BRAF- and MEK inhibitors have improved outcomes for BRAF-mutant melanoma, effective options for NRAS-mutant melanoma remain limited. Therefore, Immune checkpoint blockade targeting the PD-1/PD-L1 axis is the first-line treatment, however, 60–70% of patients do not respond, highlighting the need for new therapeutic strategies. The mevalonate pathway is an essential pathway synthesizing sterols (such as cholesterol) and non-sterol isoprenoids as well as providing intermediates for post-translational modifications (PTMs), such as protein prenylation, needed for anchoring signalling proteins, such as RAS and PD-L1, to membranes ensuring proliferation and cancer cell survival and subsequently offering a branch of immunometabolism to study in NRAS-mutant melanoma.

Aim: The aim of this study is to exploit the vulnerabilities of PD-L1/PD-L2 in context with the mevalonate pathway to study immunometabolic differences between BRAF-mutant and NRAS-mutant melanoma. Using in vitro and in vivo approaches we're proposing to give insights in the different dependency of BRAF- and NRAS-mutant melanoma on the mevalonate pathway and the connection of PD-L1 and JAK/STAT signalling. We're using mouse xenograft models based on YUMM1.7 cells (BRAF V600E) and OSUMMER.2 cells (NRAS Q61R) via subcutaneous injections in C57BL/6 mice.

Results: Using in vitro and in vivo models, we determined how modulation of pathway intermediates, cholesterol availability, and prenylation affects checkpoint expression, including JAK/STAT signalling, specifically STAT3 and STAT1. PD-L1 as well as pSTAT1 (Y701) is upregulated upon Lonafarnib (FTI) treatment in SkMel2 (NRAS Q61R) cells, whereas GGTI-2133 (GGTase-I) increases pSTAT3 (Y705) and PD-L1 in A375 (BRAF V600E) cells, but not reciprocally. PD-L2 is not affected in both cases. Simvastatin and YM-53601 are decreasing pSTAT1, pSTAT3, PD-L1, and PD-L2 regardless of melanoma subtype. Acute cholesterol depletion using Methyl-β-cyclodextrin (MβCD) did not affect PD-L1 or PD-L2, suggesting the dependency of PD-L1 on different branches of the mevalonate pathway and melanoma subtype. In vivo results are demonstrating significantly increased PD-L1 and PD-L2 levels as well as an immunogenic phenotype in the NRAS-mutant syngeneic mouse model using OSUMMER.2 cells, in comparison to BRAF-mutant YUMM1.7 derived tumours.

Conclusions: Our findings reveal subtype-specific dependencies on the mevalonate pathway through STAT1 and STAT3, supporting to target respective branches to improve immune checkpoint responses in NRAS-mutant melanoma and therefore to propose a new possible therapeutic combination therapy to anti-PD-L1treatment.

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Poster 78

Hexosamine Metabolism Controls O-GlcNAc-Dependent DNA Repair and Determines Therapeutic Response in Pancreatic Cancer

Barbara Zerbato[1], Gabriele Taverna[1], Silvia Lombardi[1], Silvia Barabino[1], Ke Deng[2], Alfonso Urbanucci[2,3], Marina La Chimia[4], Lorenzo Taglietti[5], Barbara La Ferla[5], Domenica Scumaci[4], Ferdinando Chiaradonna[1]

[1]Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy; ferdinando.chiaradonna@unimib.it
[2]Dept. of Tumor Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway
[3]Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland
[4]Dept. of Experimental and Clinical Medicine, Magna Græcia University of Catanzaro, Italy
[5]Dept. of Earth and Environmental Sciences, University of Milano-Bicocca, Milan, Italy

Background: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, with gemcitabine (GEM) resistance representing a major therapeutic challenge. A key driver is the ability of PDAC cells to efficiently repair GEM-induced DNA damage through Homologous Recombination (HR). The central role of HR in chemoresistance is validated by the observation that BRCA1/2-mutated PDAC (~10% of cases), where HR is constitutively deficient, shows marked sensitivity to DNA-damaging agents and PARP inhibitors (PARPi). Increased HBP flux sustains HR proficiency in wild-type patients via O-linked glycosylation (O-GlcNAc) of DDR components including ATM, H2AX, Chk2 and MDC1, a metabolic vulnerability exploitable to overcome chemoresistance.

Aim: Building on this HBP-HR axis, we evaluated whether FR054, a novel PGM3 inhibitor, could disrupt O-GlcNAc-dependent DDR signalling, impair HR, and thereby sensitise HR-proficient BRCA1/2 wild-type PDAC cells to GEM and PARPi.

Results: FR054 induced DNA damage and apoptosis alone and synergistically with GEM, evidenced by γH2AX accumulation and caspase-3 activation. Mechanistically, it abolished ATR-CHK1 and ATM-CHK2 checkpoint signalling while promoting ATR and ATM degradation, collapsing DDR surveillance. DSB repair reporter assay (DR-GFP) assays confirmed selective, dose-dependent HR impairment without affecting NHEJ. Strikingly, whereas olaparib alone was ineffective, FR054 synergised strongly with olaparib in HR-proficient BRCA1/2 wild-type cells, with persistent γH2AX foci and reduced BRCA1 nuclear recruitment confirming a functional BRCAness state.

Conclusions: HBP inhibition by FR054 metabolically seems to induce a BRCAness-like state, impairing HR and enhancing GEM and PARPi vulnerability in BRCA1/2 wild-type PDAC, potentially expanding targeted therapies to the majority of patients currently ineligible for PARPi.

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Metabolic Reprogramming Mediated by NF-YA Alternative Splicing: A Therapeutic Vulnerability in Colorectal Cancer

Arianna Rinaldi[1], Silvia Bellutia[1], Valentina Mularonia[1], Natalia Iseppato[1], Virginia Campani[1], Mirko Ronzio[3], Valeria Righi[2], Laura Cuoghi[1],Tommaso Martinelli[1], Oneda Cani[7], Andrea Alessandrini[4,5], Giacomo Miserocchi[6], Diletta Dolfini[3], Vincenzo Zappavigna[1], Carol Imbriano[1*].

[1]Department of Life Sciences, University of Modena and Reggio Emilia, via Campi 213/D, 41125 Modena, Italy;
[2]Department for Life Quality Studies, University of Bologna, Corso d'Augusto 237, 47921 Rimini, Italy;
[3]Department of Biosciences, University of Milan, via Celoria 26, 20133 Milan, Italy;
[4]Department of Physics, Informatics and Mathematics, University of Modena and Reggio Emilia, via Campi 213/A, 41125 Modena, Italy;
[5]CNR-Nanoscience Institute-S3, via Campi 213/A, 41125, Modena, Italy;
[6]Biosciences Laboratory, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy.

[a]These authors contributed equally

Background: Colorectal cancer (CRC) cells rely on metabolic plasticity to adapt to adverse microenvironmental conditions and sustain malignant progression. In aggressive CRC, glutamine metabolism may provide an adaptive advantage, but the mechanisms controlling this pathway remain poorly defined.

Aim: This study investigated whether NF-Y regulates glutamine metabolism in CRC and defined the contribution of the NF-YA splice variants to stress adaptation and tumor aggressiveness.

Results: Integrating NF-YA loss-of-function with transcriptomic and metabolomic analyses, we show that NF-YA controls glutamine metabolism in CRC cells. Gain-of-function approaches indicate that this function is mediated by the NF-YAl isoform. Mechanistically, NF-YAl binds the Glul promoter, inducing glutamine synthetase transcription and increasing intracellular glutamine availability. This metabolic shift enables CRC cells to tolerate oxidative stress and shear forces under glutamine-restricted conditions, supporting migration and metastatic potential. Pharmacological inhibition of glutamine synthesis, but not glutamine uptake or catabolism, reduces the survival and migration advantages conferred by NF-YAlhigh CRC cells both in vitro and in vivo. NF-YAl-driven glutamine biosynthesis remodels the tumor microenvironment by favoring M2 macrophage polarization. Glutamine released by NF-YAlhigh CRC cells drives human monocytes toward an M2-like phenotype. Consistently, patient-derived datasets support the NF-YAl–Glul–M2 axis as a predictor of CRC aggressiveness.

Conclusions: Our findings identify NF-YAl as a regulator of glutamine biosynthesis in aggressive CRC and establish glutamine synthetase as a critical effector of NF-YAl-driven metabolic reprogramming, highlighting glutamine synthesis as a targetable metabolic vulnerability in NF-YAlhigh CRC tumors.

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