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Poster Abstracts

Session 1

Poster 1 - **The natural L370F ER α Variant Confers Endocrine Resistance and Sensitivity to ATRA in Metastatic Breast Cancer Cells**

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Metastatic breast cancer (MBC) remains a major clinical challenge, particularly in estrogen receptor α (ER α)-positive patients who develop resistance to endocrine therapy (ET). While hotspot mutations such as Y537S in the ligand-binding domain (LBD) are well-characterized drivers of resistance, other ER α variants remain poorly studied. Understanding the molecular mechanisms underlying resistance in these variants is crucial for identifying novel therapeutic strategies. Here, we investigated the functional role of the L370F and E471D ER α variants, which are spatially close in the ER α structure. Stable overexpressing HEK293 cells and CRISPR/CAS9 engineered MCF-7 cells were generated and treated with 17 β -estradiol (E2), fulvestrant (Ful) and all-trans retinoic acid (ATRA) to measure ER α stability, transcriptional activity and gene expression analyses using different cellular assays and RNASeq techniques. Direct in vitro measurement of ligand binding affinity to ER α were performed using the purified full-length wild type (wt) as well as L370F and Y537S ER α . In silico structural simulations were also performed to predict the structure of the mutated L370F ER α . Senescent analyses of MCF-7 and Y537S MCF-7 cells were performed using direct measurement β -galactosidase activity in vitro and in cell lines. The L370F variant conferred resistance to Ful in terms of in vitro ER α binding, ER α transcriptional activity, receptor degradation and cell proliferation by modifying the folding of the receptor structure. Furthermore, L370F-expressing cells exhibited a hyperactive response to low doses of E2 and basally upregulated late estrogen responsive genes. Additionally, we found that both L370F and Y537S ER α variants displayed increased RAR α expression, rendering them highly sensitive to ATRA. Notably, ATRA killed L370F-expressing cells and induced senescence in Y537S-expressing cells, highlighting mutation-specific responses. Our findings expand the understanding of ER α mutations beyond known hotspots, identifying L370F as a novel mutation contributing to ET resistance and further indicate the necessity to characterize all the less-studied ER α variants found in MBC. Furthermore, we demonstrate that ATRA selectively targets MBC cells harboring L370F and Y537S mutations, suggesting its potential as a mutation-specific therapeutic agent. These results support further investigation of ATRA in clinical settings to improve treatment strategies for ER α -mutant MBC.

Poster 2 - Archaeogenomics of Italy

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Analysis of genomic datasets from modern Italian populations revealed a high degree of genetic variation comparable to that found throughout Europe. This suggests that Italian gene pools today are largely the result of evolutionary processes, including admixture, migration, and genetic drift, which primarily affect neutral alleles, as well as selection and adaptive events, which impact functional variants. High-resolution, representative data are required to disentangle the contributions of these processes, as they often occur within short timeframes, leaving similar or partially overlapping patterns of genetic variation. Such data would also enable us to infer past demographic histories and fluctuations in effective population size. However, there is currently no ancient genome reference panel available to describe Italy's genetic complexity at a high resolution. This archaeogenomics project, which focuses on Italy, began addressing this issue.

A large collection of DNA libraries from ancient Italian individuals, prepared in dedicated cleanroom facilities, was used to generate preliminary data through shallow sequencing. A pipeline specifically developed for ancient DNA analysis was used to select libraries with good complexity and excellent endogenous DNA preservation (>10%) for deep sequencing.

A total of 133 high-coverage genomes (>1X for ancient DNA data) could be produced only thanks to the joint venture with the HT National Facility of Genomics. This dataset will be analyzed using allele frequency- and haplotype-based approaches to study patterns of shared ancestry and gene flow, as well as to explore local ancestry and relationships among populations and individuals. The high-resolution data provided by 5x genomes will also allow us to investigate rare variants and population dynamics and eventually apply it to detect potential signatures of selection for the first time on ancient Italian populations.

Poster 3 - **Exploring cholesterol's role in immune checkpoint inhibitor response: identification of metabolic biomarkers and mechanisms of resistance in advanced renal cell carcinoma**

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Immune checkpoint inhibitors (ICIs) have transformed the treatment of metastatic renal cell carcinoma (mRCC) by enhancing antitumor immune responses; however, durable clinical benefit occurs in only a subset of patients, highlighting the need to identify biological determinants of response and resistance. Clinical evidence from our group shows that baseline serum cholesterol is an independent prognostic factor in patients with advanced cancer treated with ICIs, indicating a previously underexplored link between systemic metabolism and tumor immune regulation.

This project investigates how serum cholesterol availability shapes the tumor microenvironment (TME) and modulates antitumor immunity in mRCC. Through competitive access to the National Facility for Genomics, tumor samples are analyzed using 10x Genomics Visium Spatial Gene Expression HD technology, enabling high-resolution spatial mapping of tumor and immune cell populations together with their transcriptional programs. This NF-enabled approach provides an unprecedented, spatially resolved view of metabolic-immune interactions, representing a novel perspective in the study of response and resistance to ICIs. The project analyzes a cohort of 24 mRCC patients stratified by high versus low baseline serum cholesterol. To date, the first seven cases have been successfully processed and analyzed, and spatial profiling of the remaining samples is ongoing.

A key innovative aspect of this project is the integration of spatial transcriptomics with metabolic and clinical data to reconstruct cell-type-specific gene regulatory networks within the TME. Advanced bioinformatic analyses will be performed with support from the National Facility for Data Handling and Analysis, enabling pathway-level interrogation, inference of cell-cell communication networks, and identification of molecular and cellular alterations associated with cholesterol abundance and clinical outcome to ICIs.

By linking systemic cholesterol to spatially defined immune and tumor transcriptional states, this study is expected to advance understanding of immunotherapy resistance and to identify novel metabolic biomarkers predictive of ICI efficacy, as well as potential targets for combination strategies.

Access to National Facilities has been pivotal in expanding the Principal Investigator's expertise in spatial and computational biology, enabling leadership of integrative immuno-oncology research and strengthening scientific independence and national collaborative positioning.

Poster 4 - **From prenatal origins to novel therapeutic strategies targeting inflammatory pathways: leveraging pre-clinical models of Juvenile Myelomonocytic Leukemia (JMML)**

Giulia Quattrini* (1), Cristiana Barone* (1), Alessandro Muratore (1), Gloria Zambelli (1), Filipa Timóteo-Ferreira (1), Thea Milanese (1), Tiphane Durfort (1), Mahdieh Naghavi Alhosseini (1), Silvia Bombelli (3), Deborah D'Aliberti (1), Valentina Sangiorgio (2), Cristina D'Orlando (1), Raffaella Meneveri (1), Barbara Vergani (1), Rocco Piazza (1,2), Silvia Brunelli (1,2), Emanuele Azzoni (1,2)

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Juvenile Myelomonocytic Leukemia (JMML) is a rare pediatric myeloproliferative neoplasm driven by mutations in the RAS pathway, half of which arise during fetal life. Despite extensive genetic characterization, the cellular context in which JMML-initiating mutations act remain incompletely understood.

To investigate how the timing and cellular context of oncogenic KrasG12D activation shape disease initiation and progression, we developed a genetically engineered mouse model allowing activation of JMML-associated KrasG12D in defined subsets of embryonic hematopoietic stem and progenitor cells (HSPCs).

Our data show that embryonic HSPCs display differential susceptibility to KrasG12D. Introduction of the mutation in embryonic hematopoietic stem cells (HSCs) resulted in the most aggressive and fully penetrant JMML-like disease in adult mice. Analysis of prenatal stages immediately following mutation acquisition uncovered the emergence of a pre-leukemic state already evident in the fetal liver, characterized by expansion and functional alteration of HSPC compartments as well as hallmark JMML traits such as GM-CSF hypersensitivity. In addition, these fetal HSPCs showed transcriptional and epigenetic reprogramming, including metabolic rewiring and Nfkb1-driven inflammation. These data identify a previously unrecognized pre-leukemic stage in JMML, directly supporting its in utero origin.

In adult mice, KrasG12D-driven JMML was associated with a pronounced myeloid-biased differentiation program and transcriptional and epigenetic changes that highlight an enhanced inflammatory signature, such as the enrichment of inflammatory-related transcriptional factors (FOS, FOSL2), consistent with a functional role of inflammation in JMML pathogenesis. Thus, we started in vivo pharmacological modulation of inflammatory signaling targeting innate immune pathways. Initial analyses indicate measurable modulation of disease-associated inflammatory features, supporting the therapeutic relevance of this approach. To comprehensively assess treatment-associated changes, transcriptomic and proteomic analyses are currently ongoing.

Our data establish a robust experimental framework to dissect how oncogenic KrasG12D signaling intersects with developmental hematopoiesis and inflammation in JMML, providing foundation for future functional and translational studies.

Poster 5 - Exploring the molecular mechanism behind meiotic X-Y nondisjunction in mammals

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Sex chromosome aneuploidies (SCAs) are among the most common variations in the human whole chromosome copy number. One of the most common SCAs is Klinefelter syndrome (KS) (47, XXY), which, unlike whole-chromosome aneuploidies of the autosomes, is often of paternal origin [1]. KS is caused by a lack of crossover formation (CO) between XY chromosomes, followed by nondisjunction at metaphase of the first meiotic division (MI) [1]. Though XY nondisjunction can occur stochastically, aneuploidy of XY chromosomes in fathers of KS patients is significantly higher compared to the control population [2], indicating a predisposition to nondisjunction. To date, the genetics behind KS is poorly understood. In mice and humans, the formation of CO between XY occurs in a restricted homology region called the pseudoautosomal region (PAR). Whether structural variations (SVs) of the PAR and Single Nucleotide gene Variants (SNVs) of meiotic-related genes contribute to XY recombination defects is not known. Here, through the 24-G-PILOT-National Facility of Genomics, we generated a de-novo X-chromosomal optical genome map of PAR, from a mouse model of XY aneuploidy developed in our laboratory [2], to identify PAR-associated SNVs and SVs potentially predisposing to XY nondisjunction.

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Poster 6 - Ion Imaging in Neuro-Muscular Organoids (iNEMO)

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The neuromuscular (NM) junction (NMJ) is the key synapse between motor neurons and skeletal muscle fibres, essential for voluntary movement. Its structural and functional impairment is central to many NM disorders, often requiring lifelong treatment. Despite recent progress, important questions remain about the molecular mechanisms that govern NMJ development, maintenance and plasticity. In particular, emerging evidence points to noncoding RNAs and other unconventional transcripts as important regulators of muscle and motor neuron physiology, but their contribution to NM circuits requires deeper definitions. These challenges align with recent advances in in vitro modelling, such as NM organoids (NMOs), which provide innovative platforms to study the NMJ niche within a complex, human-derived architecture. In our work, NMO manipulations are combined with cell-type-specific molecular profiling and advanced imaging to map how perturbations in these regulatory layers reshape NM connectivity. We further couple these structural and molecular readouts with functional assessment on Micro-Electrode Array platforms, which allow monitoring of network activity, synaptic transmission and coordinated muscle contraction dynamics.

Through this multimodal strategy, we begin to define how noncanonical gene networks influence NMJ assembly, maturation and synaptic efficacy. Overall, our approach offers a refined, cell-type-resolved view of NM communication and opens new avenues to interrogate the noncoding regulatory landscape underlying human NMJ physiology and its vulnerability in disease.

Poster 7 - **Single-cell dissection of phenotypic/genotypic determinants of metastasis founder cells in lung cancer**

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Circulating tumor cells (CTCs) are key mediators of metastatic spread and represent promising non-invasive biomarkers for monitoring tumor evolution. However, their clinical translation is hindered by extensive phenotypic and genetic heterogeneity. Accumulating evidence indicates that only a small subset of CTCs possesses metastasis-initiating capacity. In non-small cell lung cancer (NSCLC), we previously identified metastasis-initiating cells (MICs) defined by the co-expression of CD133 and CXCR4. In the CTCNeo clinical study (INT76/21), detection of circulating MICs in patients treated with platinum-based neoadjuvant chemotherapy was associated with reduced response and poor outcome. Nevertheless, the direct link between CTC phenotype, genomic profile and metastatic competence remains largely unexplored.

Here, we applied an integrated single-cell strategy to dissect the phenotypic and genomic determinants of metastatic fitness in NSCLC. Using an established biobank of single CTCs isolated by DEPArray technology and thanks to NF Genomics access, we combined high-resolution phenotypic characterization with genome-wide copy number alteration (CNA) profiling at the single-cell level.

A total of 120 single CTCs underwent whole-genome amplification using the MALBAC scWGA Kit, followed by library preparation, quality control and low-pass whole-genome sequencing. Reliable CNA profiles were obtained for approximately 50% of samples, enabling comparative analyses of 32 MICs and 56 non-MICs from 22 NSCLC patients.

Groupwise comparisons identified a MIC-specific CNA profile, with 49 alterations significantly enriched in circulating MICs, predominantly chromosomal losses involving key oncogenic pathways such as WNT/ β -catenin, TP53, and MYC. MIC-CTCs also displayed significantly higher genomic instability, characterized by extensive chromosomal rearrangements defined by large-scale state transition scores. Importantly, distinct CTC-CNA patterns were also associated with disease progression and survival, supporting the development of a CNA-based classifier to identify NSCLC patients at high risk of relapse.

Overall, access to NF Genomics provided novel insights into the phenotypic/genotypic determinants of metastatic competence, advancing single-cell biomarker discovery for personalized therapies

Poster 8 - Drug screening on NDRG1, a protein involved in nickel-driven lung cancer

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Drug discovery has traditionally focused on well-folded proteins with stable three-dimensional structures. However, the urgent need to expand the range of therapeutic targets has shifted attention toward intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs), which play key roles in cancer and other complex diseases. Although their structural plasticity poses major challenges for conventional drug-design strategies, it also represents a largely untapped opportunity for innovation. This study focuses on the intrinsically disordered C-terminal region of the human protein NDRG1 (hNDRG1^{*C}), a stress-responsive protein with context-dependent roles in tumorigenesis and particular relevance in lung cancer. hNDRG1^{*C} is characterized by high conformational flexibility and undergoes post-translational modifications such as phosphorylation. It interacts with metal ions, including Ni(II), and with negatively charged lipid membranes (DMPG). These features make hNDRG1^{*C} a challenging but attractive target for ligand discovery. The main objective of this work was to assess the feasibility of identifying small molecules capable of selectively binding hNDRG1C in both its unmodified and phosphorylated states, thereby evaluating its potential as a druggable hotspot within an IDP. An innovative experimental strategy such as microscale thermophoresis (MST) was employed to perform a focused compound screening. Despite the limited size of the initial compound library, two distinct binders were successfully identified. Although further structural characterization using techniques such as NMR spectroscopy, mass spectrometry and thermal shift assay is required, these results demonstrate that ligand discovery targeting IDPs and IDRs is achievable and provide one of the first experimental validations of hNDRG1C as a molecular target.

Access to the Human Technopole National Facility was essential for the success of this project. The availability of advanced instrumentation enabled low-concentration, high-sensitivity measurements that would not have been feasible in the home laboratory and directly supported the development of this innovative approach.

Poster 9 - **Dissecting the role of tumor-associated macrophages in breast cancer angiogenesis.**

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Breast cancer is among the most frequently diagnosed cancers worldwide and remains the second leading cause of cancer-related mortality in women. A critical process underlying breast cancer growth and metastasis is vascularization, which enables sustained tumor expansion and dissemination. Under physiological conditions, macrophages are key anti-tumor immune cells and contribute to angiogenesis by promoting blood vessel anastomosis. In cancer, however, macrophages undergo phenotypic reprogramming into tumor-associated macrophages (TAMs), adopting a tumor-tolerant, pro-angiogenic M2-like state that supports tumor growth. The stromal and macrophage compartments of the tumor microenvironment (TME) are major sources of pro-angiogenic and pro-tumorigenic mediators, fostering tumor vascularization, progression, metastasis, and adaptive resistance to therapies. Consequently, TAMs and their role in TME remodeling and angiogenesis represent promising therapeutic targets.

We demonstrate that stage-dependent depletion of macrophages in a mouse model of breast cancer induces opposite effects on tumor growth and progression. Ablation of physiological macrophages at early stages of tumor development resulted in a marked worsening of disease, whereas late ablation of polarized TAMs led to a strong reduction in tumor growth and vascular dysregulation. Based on these findings, we performed transcriptional profiling of TME evolution at single-cell resolution of murine breast cancer to investigate the temporal dynamics of TME remodeling and the specific contribution of TAMs to tumor angiogenesis.

Through collaboration with the Human Technopole FACS facility and the National Facility for Genomics, we generated an enriched single-cell dataset capturing the molecular signatures and temporal evolution of TME populations, moving the focus from cancer cells to stroma cells and distinguishing early anti-tumoral from late pro-tumoral TAM signatures. This analysis provides insights into TAM pathological transition and their cross talk with endothelial cells. We also identified how TAMs loss reshapes TME composition.

This dataset may reveal novel targets for anti-angiogenic therapies in breast cancer.

Poster 10 - **Molecular dynamics of the egg membrane during fertilization**

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Fertilization is the culminating event of sexual reproduction, which, in mammals, involves the fusion of sperm and egg to form a single, genetically distinct organism. Although fertilization is central to the propagation of many species and despite having a detailed cellular description, the molecular mechanisms remain poorly understood.

The identification of the Izumo1-Juno binding pair (doi: 10.1038/nature13203) provided a mechanism to explain how sperm and egg recognize each other, and recent evidence suggest they might form a complex with other membrane proteins (doi: 10.7554/eLife.93131), nonetheless the dynamics of these events in the context of cell membranes is still unclear.

This project introduced the use of super-resolution microscopy to investigate the events occurring on the egg cell membrane at the time of fertilization. The aim was to bridge the gap between existing descriptive knowledge of sperm-egg interaction and the molecular mechanisms governing fertilization. Data obtained with conventional microscopy indicate that a significant reorganization of the egg membrane happens during fertilization therefore we applied STED (Stimulated Emission Depletion) microscopy to uncover unprecedented details of the sperm-egg interaction, ultimately contributing to unravel the mysteries of fertilization at the nanoscale level.

Access to the National Facilities made it possible to test reagents and establish experimental conditions suitable for STED microscopy. Working with the mouse egg presents intrinsic challenges: it is an extremely limited cell type, unusually large (~ 80 μm), maintained in suspension throughout fertilization and imaging. STED microscopy provided a clear improvement in resolving JUNO localization; however, simultaneously staining the oolemma and JUNO-binding partner, IZUMO1, proved considerably more difficult than anticipated. Another, unexpected challenge was distinguishing the single fertilizing sperm from the many others attached to the oolemma—an issue that cannot be solved using standard cell markers.

Overall, access to the NF was essential for assessing the feasibility of this imaging technique and for identifying the adjustments required to pursue a more refined analysis of fertilization.

Poster 11 - **Definition of Top1cc-induced mutational signatures in cancer and neurodegenerative disease cell models**

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DNA topoisomerases regulate DNA topology during fundamental processes such as transcription and replication. Topoisomerase I (Top1) forms transient Top1-DNA cleavage complexes (Top1ccs), which can be stabilized by antitumor Top1 poisons such as camptothecin (CPT) analogs. This stabilization increases Top1cc levels, perturbs RNA and DNA synthesis, and promotes ubiquitin-dependent Top1 degradation. We recently showed that CPT-stabilized Top1ccs enhance transcription-replication conflicts in cancer cells, triggering DNA double-strand breaks at highly transcribed genes within early-replicating regions and leading to chromosome instability¹. In addition we know that mutation or deletion of TDP1, an enzyme specifically involved in the repair of irreversible Top1ccs, delay the repair of double-strand DNA cleavage induced by Top1ccs in cell models². We therefore hypothesize that persistent Top1ccs may substantially reshape the cancer genome by promoting specific mutations and rearrangements at Top1cc-associated damage sites. Because the mutational footprint of Top1 poisons remains poorly defined, we performed an exploratory analysis of mutational signatures in patients with metastatic colorectal cancer whole-genome sequencing data from the Hartwig Medical Foundation cohort³. We quantified mutational signature contributions and, among insertion/deletion signatures, only de novo ID5 (unknown etiology) showed specificity for Top1 poison treatment. To validate these observations, we generated WGS data from the HCT116 colorectal cancer cell line treated with CPT and compared it with untreated control. In this model, we detected a single indel signature (COSMIC ID9) closely matching the cohort-associated pattern, characterized by 1 bp deletions at C/T nucleotides. The NF project has extended the analyses to other cancer cells and TDP1 KO cell lines. We have also analyzed genome sequences from other cancer cell models and from cells with a specific TDP1 mutation, known to cause the rare SCAN1 disease². The analyses of these data will be presented at the meeting. Up to now, our findings nominate a Top1 poison-associated indel signature in colorectal cancer and support a link between Top1cc-driven genome stress and distinctive mutational outcomes. The data accumulated during the NF project will be critical to refine the signature definition, assess dose/exposure dependence, and test mechanistic models connecting Top1cc stabilization to localized indel formation and structural variation.

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Poster 12 - Cryo-EM reveals mouse serum albumin flexibility

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In this work, we present the results obtained through the 24-SB-PILOT call at Human Technopole (HT) regarding the project "Unveiling the structural details of the mouse serum albumin recycling process". The detailed three-dimensional structure of the mSA-mFcRn complex is urgently required in the context of structure-based drug design. Indeed, despite mouse being the primary model organism used to evaluate albumin-based therapeutics, and serum albumin (SA) being well documented to influence drug bioavailability and pharmacokinetics, its molecular structure has still not been experimentally determined. It is well known that albumin extends drug half-lives in several animal models; but for most of these drugs the albumin binding site remains elusive. Moreover, to reach prolonged half-life, drug binding sites must not interfere with the cognate binding mode of FcRn. In contrast to the human hSA-hFcRn complex, the structural details of the mSA-mFcRn contact site are still unknown. The elucidation of residues engaged in the receptor binding and thus not available for other molecules capture, is essential to identify which residues can be targeted by the drug. Here, we present the cryo-EM structure of mSA in complex with a megabody (MgbAlb1) selected by the VUB Institute of Bruxelles to be cross-reactive for both mouse and human SA (De Felice et al., 2024). Using the DinaMight software implemented in RELION, we reveal mSA conformational heterogeneity and flexibility, resolving multiple atomic models below 3 Å resolution, spanning intermediate states between the classical open and closed conformations described by X-ray crystallography for hSA (De Simone et al., 2021). Notably, mSA domain III, which has been described in the literature as the putative one involved in mFcRn binding, displays the highest degree of flexibility. To stabilize the complex, we decided to perform cross-linking mass spectrometry, a technically challenging approach due to the acidic pH required for the interaction between the two species. Here, we present the first preliminary data obtained, along with the ongoing and future optimization strategies.

Poster 13 - **Genome engineering strategy to validate the role of the Hippo pathway as a novel regulator of dendritic cell physiology**

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Dendritic cells (DCs) are bone marrow-derived cells that play a pivotal role in the physiology of the immune system. They act as primary sentinels, and they traffic through areas characterized by different oxygen tensions. Hypoxia is a feature of inflamed tumor tissues and lymphoid organs, including bone marrow and lymph nodes where DCs exert their main functions. Thus, the modality by which DCs adapt to hypoxia is important to guarantee the quality of the immune response. Indeed, previous reports indicate that hypoxia influences DC physiology cells through adaptive molecular processes involving HIF-1 to ensure their physiological function. However, the multitude of pathways and factors that might regulate DC physiological functions is not fully understood. It has been reported a correlation between hypoxia signaling and the Hippo pathways in several cellular models, but not in DCs. Thus, we are proposing the Hippo pathway as a novel potential regulatory mechanism that might be crucial for DC physiology under hypoxia in the context of inflammatory processes and autoimmune diseases. Preliminary results indicate an association between the expression of YAP, the master regulator of the Hippo pathway, with inflammatory pathways, such as MAPK p38, and cytokines including IL-1 β . This suggests that the Hippo pathway may be involved in novel molecular mechanisms regulating DC functions, with significant implications in the immune system homeostasis and inflammation.

Poster 14 - **Gut-derived immune stimulation remodels the choroid plexus to reinforce CNS barrier defense**

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The choroid plexus (ChP) has recently emerged as a critical immunological interface between the central nervous system (CNS) and the periphery. In this study, we investigated how gut-derived immune signals shape ChP immunity and promote long-term immune adaptation. Using complementary preclinical models, including systemic *Salmonella Typhimurium* infection and mucosal vaccination with an attenuated *S. Typhimurium* Δ aroA strain, we examined the impact of intestinal immune cues on ChP immune function. We identified a previously unrecognized presence of IgA within the ChP. Following intravenous infection, the ChP displayed strong resistance to microbial colonization, supporting its role as a protective “filter” against blood-borne pathogens. Notably, mucosal immunization led to the incorporation of bacterium-specific IgAs, generated in the gut, into the ChP. Moreover, multiparametric flow cytometry revealed that mucosal immunization induced sustained remodeling of the ChP immune landscape, characterized by a strong redistribution of both stromal and epiplexus-resident BAM populations, together with neutrophil mobilization. Importantly, this immune reshaping persisted over time and correlated with enhanced protection upon reinfection, providing functional evidence that intestinal vaccination imprints a memory-like state in ChP innate immune cells, reminiscent of trained immunity. To formally assess this phenotype, we are employing integrated single-cell multiome gene expression and ATAC-seq analyses. This approach will allow us to define stable transcriptional programs and epigenetic reconfiguration in ChP BAMs, thereby providing molecular validation of the functional adaptations observed. We anticipate that this analysis will uncover persistent changes in chromatin accessibility linked to innate immune and antimicrobial pathways, directly connecting epigenetic remodeling to the enhanced protective capacity of ChP BAMs. Collectively, our findings establish the gut-ChP immune axis as a key integrator of adaptive and innate mechanisms that reinforce the CNS barrier against infections, while simultaneously highlighting the potential risk that mucosal stimulation may also promote chronic neuroinflammation.

Poster 15 - **Dissecting TGF- β 1 Signaling and Microglia-Organoid Co-Culture Dynamics Using TGFBR2-Knockout hPSCs**

Anna Cascio (1)*, Stefania Giussani (2), Carlo Manenti (2), Paolo Vaccari (1), Sara Ferro(1), Jose Davila-Valderrain (2), Oliver Harschnitz (2), Veronica Krenn (1)

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Human brain development is exquisitely sensitive to immune influences. In particular, microglia—the brain’s resident immune cells—play essential roles in development and disease, yet many of their human-specific features are poorly captured in animal models, limiting our understanding of their impact on brain development and pathology.

Recent advances in microglia-containing brain organoids, also termed neuroimmune assembloids (NIA), represent a major conceptual and technological innovation, providing unprecedented opportunities to model human neuroimmune interactions in a three-dimensional, developmentally relevant context. However, the molecular pathways governing human microglial states within these systems remain incompletely understood, constraining their translational potential.

Using human NIA, we investigated the role of TGF- β signaling, an evolutionarily conserved pathway implicated in neurodevelopmental disorders and neurodegeneration. We found that TGF- β 1 supplementation robustly reprograms human microglial states in NIA, shifting their transcriptional profile from metabolic toward immune-associated programs. Leveraging access to National Facilities (NF), we sought to establish causality by generating TGF- β signaling-deficient microglia via bi-allelic knockout of the TGF- β receptor type II (TGFBR2) in human pluripotent stem cells integrated within NIA cultures. This innovative genetic strategy enables us to disentangle cell-autonomous microglial TGF- β signaling from broader tissue effects for the first time in a human organoid system.

Scientifically, this work helps us define how TGF- β signaling shapes human microglial identity during early brain development. More broadly, it validates NIA as a mechanistically tractable neuroimmune organoid platform, providing essential tools to dissect microglial influences on human brain development and disease.

Poster 16 - **Tracing gut microbiome traits linked to the Mediterranean diet from Roman times to the present**

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The archaeological site of Pompeii represents a unique opportunity to investigate daily life in the Roman era, as the eruption of 79 AD preserved houses, urban infrastructure, and human remains in an exceptional state. A recent excavation uncovered a latrine from the House of Obellio Firmo (late Augustan period) that had remained sealed and uncontaminated since Roman times, allowing direct access to ancient fecal material. In this study, we characterized traces of the ancient gut microbiome and dietary components using shotgun metagenomics, with ultra-deep sequencing performed at Human Technopole, enabling the reconstruction of ancient intestinal bacterial genomes. Analyses were conducted in dedicated clean laboratories, and the ancient origin of the DNA was validated through characteristic post-mortem damage patterns using HOPs. We detected authentic ancient DNA from human gut-associated bacteria, as well as plant-derived DNA from foods such as onions, olives, sage, dates, wheat, jujube, and grapes, in agreement with historical reconstructions of the Roman diet. Finally, we compared the reconstructed gut microbiome profile of the Pompeii individual with that of modern Italians adhering to a Mediterranean diet, highlighting both conserved and divergent microbiome traits. Overall, our findings provide direct evidence of the long-term association between dietary practices and gut microbiome composition, tracing features linked to the Mediterranean diet back to Roman times and offering new insights into the co-evolution of human diet and intestinal microbiomes in Italy.

Poster 17 - **Using optical tweezer and confocal imaging to visualize and characterize the dynamics of RAD51 binding to DNA abasic sites**

Anna De Antoni*(1), Joanna Andrecka (3), Vincenzo Costanzo (1,2)

(1) IFOM ETS, (2) University of Milan, (3) Human Technopole

Abasic or apurinic/apyrimidinic (AP) sites, are among the most frequent endogenous DNA lesions. They arise spontaneously and as obligatory intermediates of base excision repair (BER). When an AP site is encountered during replication, the fork faces a nasty dilemma: if the lesion is ignored, replicative polymerases stall and the fork becomes unstable; if the lesion is processed at the wrong time or in the wrong structural context, it can be converted into a strand break and, ultimately, a double-strand break. Therefore, it should exist a mechanism capable of stabilizing the lesion-containing intermediates transiently and also allowing productive repair.

We have recently shown [1] that the homologous recombination proteins RAD51 specifically recognizes and binds AP sites during replication, protecting them from nuclease mediated processing.

Here I generated long DNA molecules bearing AP sites in defined architectures, compatible with optical tweezers manipulation, microfluidic exchange, and fluorescence imaging and developed a system to visualize RAD51's interaction with AP sites on individual DNA molecules capturing real-time dynamics. This system allows to go beyond the static observation that "RAD51 binds AP-DNA" and can instead define how AP lesions influence RAD51 filament nucleation and dynamics.

In cells, AP sites are rarely isolated lesions in relaxed duplex DNA, but they arise near moving forks, within ssDNA gaps, at junctions, and under competitive binding with the single strand binding protein RPA, mediators, and nucleases. Therefore the system will be further developed so that to reconstruct replication-relevant lesion contexts, including AP sites within ssDNA gaps and at ssDNA/dsDNA junctions with or without RPA, and mediator systems; furthermore we can extend the system to BER-refractory contexts such as G-quadruplexes and R-loops, where clustered AP lesions are expected to persist and require specialized processing. The final goal is to derive quantitative rules for lesion fate as a function of DNA architecture, mechanical state, and repair ensemble composition.

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Poster 18 - **Characterization of meningioma by Whole Genome Sequencing to identify new molecular subtypes and biological drivers**

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Meningiomas are the most common intracranial tumors in adults, representing about 40% of all brain tumors. While most meningiomas are benign and manageable with surgery and/or radiotherapy, the classification and prognosis of higher-grade tumors remain challenging due to subjective histopathological grading and significant observer-dependent variability.

To fill this gap, thanks to a project awarded by the NF 24-G-PILOT Call, we performed whole genome sequencing (WGS) on an internal cohort of 100 fresh-frozen meningioma samples across World Health Organization (WHO) grades 1-3 with well-annotated clinical, histopathological, and follow-up data. The aims of the study are: (I) uncover recurrent genomic alterations, (II) define molecular subtypes, and (III) explore their biological and clinical relevance. The cohort size and availability of associated metadata enable robust, clinically relevant insights, offering significant contributions into meningioma tumor biology and clinical management.

By identifying novel biomarkers and potential therapeutic targets, our work seeks to refine patient stratification and identify actionable molecular features thus advancing precision medicine in neuro-oncology. This project will be further pursued and validated through biochemical assays, and its findings will be integrated with additional layers of multi-omics analyses (i.e., transcriptomics, epigenomics, proteomics, and metabolomics) to provide a comprehensive, multi-level understanding of meningioma biology.

Access to the National Facility (NF) has been pivotal for the realization of this study, particularly for providing large-scale sequencing and bioinformatics infrastructures. The NF provides state-of-the-art genomics and computational platforms, enabling high-quality WGS data generation and rigorous analytical workflows. Continuous feedback with experts from the NF team has guided experimental design and data analysis, ensuring scientific rigor and reproducibility. Moreover, the support provided through the NF call has allowed us to optimize resources and save funding, making the project both cost-effective and technically feasible. Overall, the 24-G-PILOT Call has been essential in advancing our expertise in large-scale, genome-driven neuro-oncology research.

Poster 19 - **Discovery of the interactome in walled forms Apicomplexa parasites by a co-fractionation mass spectrometry-based method approach.**

Despoina Koukouli*, Francesco Carbonetti, Elisabetta Pizzi, Chiara Currà

Istituto Superiore di Sanità

Innovative approaches and originality

Plasmodium and Toxoplasma are two of the major health and socioeconomic importance worldwide Apicomplexa. During the life cycle, both parasites pass through walled forms still poorly understood also due to difficulties to manipulate. The project developed at HT-NFs aimed at the set-up of the co-fractionation mass-spectrometry (CF-MS) based method for the detection in high throughput, under native cellular conditions, of proteome-wide protein-protein interaction in the rodent model Plasmodium berghei oocysts collected from mosquito midguts and in Toxoplasma gondii brain cysts collected from mice. CF-MS is a flexible and powerful method to detect physical associations of proteins yet not been applied in walled forms of Apicomplexa.

Scientific impact and contribution to the field

Plasmodium, the main agent causing malaria, causes 250-400 million infections and approximately half a million deaths every year. Toxoplasma gondii, which causes severe congenital disease and deaths in immunocompromised individuals, is one of the most widespread human pathogens, infecting up to one-third of the world population. Targeting the cyst stage will support approaches: 1) to block Plasmodium development inside the mosquito. In the future results could be used to make a real impact in the field by "curing" the infected mosquitoes; 2) to study interactome networks established during tachyzoite-to-bradyzoite switch and cyst formation, a new vision on the future treatment of Toxoplasma chronic phase.

Impact of Access to NF on PI's research

The outcome of the NF preliminary study has been taken as starting point in the development of two PhD projects, one conducted by Ms Despoina Koukouli in bioinformatics and the other by Mr Francesco Carbonetti in the molecular biology of Toxoplasma brain cyst. The PI, who recently joined the Istituto Superiore di Sanità, got also an important starting point benefit to develop the projects and her career within the parasitology unit

Poster 20 - **HSPB3 as a candidate to promote postnatal differentiation of motor neurons and skeletal muscle cells, maintaining the neuromuscular junction plasticity**

Samuele Crotti (1), Michela Mochi (2), Valentina Secco (1), Francesco Antoniani (1), Laura Mediani (1), Margherita Medici (2), Beatrice Silvestri (2), Alessandro Rosa (2), Serena Carra (1)

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Neuromuscular diseases such as amyotrophic lateral sclerosis (ALS) and distal hereditary motor neuropathies (dHMNs) are clinically distinct but share converging pathogenic mechanisms, including impaired axonal transport, and early neuromuscular junction (NMJ) dysfunction. NMJ pathology precedes motor neuron (MN) loss and is accompanied by cycles of denervation and reinnervation, whose efficiency declines with aging. These observations highlight the importance of understanding molecular regulators of postnatal neuromuscular differentiation, regeneration and plasticity.

This project focuses on HSPB3, a small heat shock protein whose rare genetic variants are associated with dHMNs and congenital myopathy with neuropathic features. HSPB3 rare variants have been suggested to exert a negative prognostic effect in ALS. Yet, HSPB3 physiological role in the neuromuscular system remains poorly defined. Using human induced pluripotent stem cell (iPSC) lines lacking HSPB3 or carrying disease-linked variants (R116P and Y118H), we generated motor neurons, skeletal muscle cells, neuromuscular co-cultures and neuromuscular organoids (NMOs). Our preliminary data show that HSPB3 promotes MN maturation and dendritic arborization, supports skeletal muscle differentiation and is required for proper NMJ development.

To elucidate the underlying mechanisms, in collaboration with the HT, we applied combined single-cell multiome ATAC-seq and gene expression profiling to day-50 NMOs to determine how HSPB3 loss or mutations reshape chromatin accessibility and transcriptional regulatory networks during neuromuscular development. Integrative epigenomic and transcriptomic analyses will clarify how HSPB3 indirectly modulates promoter accessibility and local chromatin remodeling.

Together, this work will help elucidating how HSPB3 contributes to the regulation of postnatal neuromuscular differentiation, NMJ plasticity and, potentially, regeneration. The results will provide a mechanistic framework for a better understanding of HSPB3 physiological function and association with neuromuscular disease.

Poster 21 - **SIP - Hidden Structure and Innervation of the mosquito Proboscis revealed by volume electron microscopy**

Marta Villa (1), Paolo Gabrieli (1)

UNIVERSITY OF MILAN

What is the neuronal organization of the labrum in *Aedes albopictus*?

This question inspired an ultrastructural analysis of the labrum using transmission electron microscopy (TEM) on resin-embedded samples, leveraging the electron microscopy service provided by Human Technopole.

Image acquisition was hampered by technical challenges intrinsic to the nature of the sample: the proboscis, being highly enriched in chitin, allowed only the collection of low-magnification images, enabling visualization of micrometer-scale features.

Nevertheless, a critical evaluation of the issues encountered proved highly informative, leading to a targeted revision of the sample preparation protocol. In particular, the importance of a prolonged post-fixation in osmium tetroxide emerged, together with the need to ensure slow and gradual resin infiltration into a structure that, far from being isometric, is organized as an extremely thin stylet.

Despite the initial technical limitations, the data clearly reveal the presence of specific sensilla localized in the most distal portion of the proboscis. This finding represents a key outcome, as it lays the conceptual and methodological groundwork for future investigations into the neuronal organization of this structure. The progressive understanding of the critical aspects of sample preparation, developed through the suggestions provided by the facility, points toward a deeper exploration of the question that motivated this study and would allow both the data already acquired and those potentially obtainable through future investigations, contributing significantly to the advancement of knowledge on the structure of the *Aedes albopictus* proboscis.

Poster 22 - **MASTERMIND: huMan cArdiac microtiSsues to capTure Epigenetic and tRanscriptomic Modifications IN carDiolaminopathies**

Natasa Dalinac* (1), Viviana Meraviglia (2), Mozhgan Seyedeh (1), Federica D'Ettore (1), Amparo Guerrero Gerboles (1), Miena Bellin (1,2)

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Mutations in the LMNA gene cause cardiac laminopathies, a rare form of dilated cardiomyopathy (LMNA-DCM) that leads to heart failure through a combination of impaired nuclear mechanoresistance, lamin A/C signaling and chromatin organization. Currently, there is no effective treatment for LMNA-DCM, hence, there is an urgent need to capture the multifaceted molecular and cellular mechanisms behind underlying LMNA-DCM pathological changes.

Innovation: Together with NF Genomics we successfully performed scATACseq and scRNAseq of hiPSC-derived 2D monocultures (cardiomyocytes, cardiac fibroblasts, endothelial and epicardial cells) and mature 3D cardiac microtissues to explore the tightly related link between chromatin remodeling and transcriptional changes caused by mutations in the LMNA gene. This will allow us to investigate cell-type specific and maturation specific contribution to LMNA-DCM at single-cell resolution in the framework of the DHA MASTERMIND project (ID 2190056) supervised by the National Facility for data handling and analysis.

Scientific impact: Here, we are generating a single-cell atlas of the chromatin organization, and its tightly related transcriptional landscape derived from transcriptionally mature hiPSC-derived cardiac microtissues (CMTs) but also non-cardiomyocyte cells, recently identified as contributors to LMNA-DCM. Investigating the intricate molecular mechanisms underlying LMNA-DCM will allow a better understanding of the disease and development of new therapies on a patient-to-patient basis.

Impact of NF Access: Our collaboration with NF Genomics will support the results obtained within the MSCA funded postdoctoral fellowship to Natasa Djalina, a MASTERMIND team member. Specifically, the dataset will be a vital component of our multi-omics screening and will be correlated with proteomic and 3D genomic outputs to establish a molecular cause behind functional cardiac impairment in the LMNA-DCM model. Additionally, the MASTERMIND dataset will facilitate publication in a high impact scientific journal and thereby strengthen the research portfolio of the team's young investigators.

Poster 23 - **Confocal and super-resolution microscopy to study neuron-microglia cross-talk in a genetic model of Autism Spectrum Disorder**

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Microglia are resident immune cells that contribute to brain homeostasis throughout life. Dysregulation of microglial function has been shown to alter synaptic pruning, brain circuitry, and neuroinflammation, which in turn can contribute to the development of Autism Spectrum Disorder (ASD), a neurodevelopmental disorder with a multifactorial etiology. The cytoplasmic FMR1-interacting protein 1 (CYFIP1) gene is linked to ASD and is enriched in microglia, where it has been shown to regulate phagocytosis and cell migration. Recently, microglial contactomics with neuronal somatic and synaptic compartments has emerged as a novel mechanism for controlling neuronal activity; however, its contribution to neurodevelopment has not yet been explored.

Thanks to access to the Light Microscopy NF Facility, we studied microglial density, morphology, and contactomics in the cortex of young mice heterozygous for *Cyfp1* (*Cyfp1*^{+/-}) under basal conditions and following immune challenge. Cortical sections stained with combinations of antibodies against Iba1 and P2Y12 (microglial markers), Kv2.1 (a neuronal soma marker), and vGLUT1 and vGAT (markers of glutamatergic and GABAergic synapses, respectively) were acquired using both confocal and STED microscopy. Image analysis revealed a consistent increase in microglial density and arborization in *Cyfp1*^{+/-} cortices, but reduced somatic contacts compared to control mice. Moreover, 24-hour immune stimulation with lipopolysaccharide (LPS) increased microglial density, with negligible morphological changes, and reduced somatic contacts in control mice. In contrast, LPS treatment rescued microglial morphology in *Cyfp1*^{+/-} mice without significantly altering microglial density or neuronal somatic contacts.

The resources and excellent technical support provided by the NF staff greatly enhanced our research, generating robust data that will be included in a manuscript for submission to a high-impact peer-reviewed journal. This work is expected to have a significant impact on both the principal investigator's visibility within the international microglia research community and the early-career researcher involved in the project.

Poster 24 - **Structural elucidation of the mitochondrial Complex I-ACAD9 assembly using cross-linking mass spectrometry and cryo-EM**

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Acyl-Coenzyme A dehydrogenase family member 9 (ACAD9) is a dual-function mitochondrial protein involved in fatty-acid β -oxidation and Complex I (CI) assembly. Although ACAD9 is known to interact with assembly factors during CI biogenesis, its potential association with the fully assembled and catalytically active enzyme has remained unresolved, as the transient nature of this interaction poses significant technical challenges. Here, we present an integrated biophysical and structural analysis of the interaction between ACAD9 and mature CI, made possible through access to the Human Technopole National Facilities (NF). Size-exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) demonstrates the formation of a stable 1:1 complex, while microscale thermophoresis (MST) reveals a low-micromolar binding affinity. Crosslinking-mass spectrometry (XL-MS) identifies interaction surfaces; finally, cryo-electron microscopy (cryo-EM) experiments performed at the NF provided direct structural insight into the intact ACAD9-CI assembly and enabled three-dimensional contextualization of the crosslinking data. The combined use of cryo-EM, quantitative biophysics and structural proteomics represents an original approach to study transient mitochondrial protein interactions. Access to the NF was essential for the successful execution of this work, providing state-of-the-art cryo-EM and mass-spectrometry infrastructure, specialized technical expertise and an interdisciplinary research environment that fueled new ideas and fostered collaborations with HT team members. These findings broaden the functional landscape of CI interactomics and underscore the critical role of shared national infrastructures in enabling original discoveries in mitochondrial structural biology.

Poster 25 - **Plasma Extracellular Vesicle RNA as predictive biomarker of immunotherapy outcome in a pan-cancer setting**

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The mechanism of action of immunotherapy-based cancer treatment with immune checkpoint inhibitors (ICI) is based on the reactivation of anti-tumor immune responses and has dramatically improved standard of care. However, lack of critical information about meaningful biomarkers guiding the choice and timing of ICI continues challenging patient outcomes. The identification of unique features related to ICI-mediated immune activation, clinical response, toxicity and efficacy might contribute to guide personalized therapy and achieve long-term clinical benefit. Extracellular vesicles (EVs) are nanometer-sized lipid-bilayer delimited particles, released by all cell types, with a cargo of lipids, proteins and nucleic acids, which circulate in body fluids. The functional involvement of EVs in immune intercellular cross talk suggests their composition and changing landscape might have strong implications for biomarker development. We obtained preliminary data using a nanoscale RNASeq to investigate if plasma EV RNA features might discriminate patient responders from non-responders to ICI therapy. Here, we aim to validate preliminary results in a larger cohort of patients affected by melanoma, lung carcinoma, renal cell cancer undergoing standard of care ICI-based therapies. Our study design includes an earlier time-point (4 weeks) to identify an on-therapy biomarker before first clinical reassessment. Comprehensive analysis of EV RNA data in concert with available clinical, peripheral blood cellular immune profiling, soluble circulating factors data will enable reaching our goal. We expect to identify a pan-cancer predictive biomarker of ICI response/resistance and related pathways shared by the different cancer types. Assessment of ICI-associated features at an early actionable on-therapy time point anticipating clinical reassessment will support personalized therapy guidance and adjustment. Results will pave the way to translation from bench-to-bedside of the plasma EV RNA biomarker.

Poster 26 - **GLUT1 Dysregulation: From Mechanisms to Clinical Implications**

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Glucose transporter 1 (GLUT1), encoded by the SLC2A1 gene, mediates basal glucose uptake in most cell types and is highly expressed in endothelial cells of the blood-brain barrier (BBB), where it's essential for cerebral energy homeostasis and barrier integrity. GLUT1 dysfunction underlies rare neurological disorders, such as GLUT1 deficiency syndrome (GLUT1 DS), and contributes to complex diseases including cancer and diabetes. Despite its critical role, GLUT1 trafficking dynamics in human BBB endothelial cells remain poorly characterized, limiting our understanding of how metabolic state, genetic variants, or regulatory mechanisms affect glucose transport and BBB function.

We performed preliminary experiments through transient transfection to characterize the subcellular localization and trafficking of wild-type protein and some missense mutants causing GLUT1 DS, also in response to changes in metabolic conditions. We disclosed that some of them mislocalized to intracellular compartments, such as lysosomes or early endosomes, and fail to respond appropriately to metabolic regulatory signals, suggesting new mechanisms of molecular pathogenesis.

To gain insight into these processes, overcoming limitations associated with overexpression systems, we aim to generate a human BBB model based on hCMEC/D3 cell line expressing endogenously GFP-tagged GLUT1 via precise CRISPR/Cas9 genome editing, thus preserving native expression levels and regulatory mechanisms. This model will allow direct correlation of GLUT1 distribution with glucose transport efficiency, regulation factors, and BBB integrity, providing new mechanistic insights into both rare and complex diseases.

Access to the Human Technopole National Facility is pivotal for the success of this project providing specialized expertise, infrastructure, and validated workflows for high-precision genome editing, substantially enhancing the PI's research capabilities. This access allows the timely generation of a BBB model that can be used as a versatile platform, even integrated in advanced 3D systems, for future translational studies and drug screening to better understand glucose metabolism in physiological and pathological conditions.

Poster 27 - **The genomic profile of a captive colony of baboons, a model organism in biomedical research**

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Baboons (*Papio* spp.) are widely used as biomedical models due to their physiological and genetic proximity to humans, yet the genomic basis of phenotypic, regulatory, and behavioural variation within and across species remains poorly understood. This project addresses this gap by generating and analysing whole-genome sequence (WGS) data from a well-characterised captive population of *Papio hamadryas*, providing a framework to investigate the genomic architecture of gene regulation, demographic history, and mating behaviour.

Through access to the National Facility for Genomics, we generated high-quality WGS data from individuals belonging to a captive baboon colony housed at the Ravenna Zoo Safari (Italy). Founded in 2013 from a limited number of captive founders and now comprising approximately 60 individuals, this colony offers a unique opportunity to study genetic variation, kinship, and reproductive dynamics in a controlled yet biologically realistic setting. Using genome-wide variants, we reconstructed pedigree structure, quantified relatedness, and characterised population diversity within the broader *Papio* phylogenetic framework. PCA and ADMIXTURE revealed clustering consistent with shared ancestry and affiliation with wild *P. hamadryas* populations, alongside limited admixture with *P. anubis*.

Importantly, pedigree reconstruction and relatedness analyses allowed us to explore multigenerational mating patterns and mating-success variability among individuals, highlighting the impact of mate-choice mechanisms on inbreeding. These results establish the colony as a powerful model to investigate from an evolutionary perspective how demographic constraints and mating preferences shape genomic diversity in primates.

Beyond population structure, the generated genomic resource provides the foundation for future investigations of gene regulation. The availability of WGS data from a colony of this size enables high-resolution mapping of expression quantitative trait loci (eQTLs) and allele-specific expression, overcoming limitations of RNA-seq-only approaches. In addition, future integration of DNA methylation data will allow the exploration of epigenetic mechanisms contributing to variation in gene expression and reproductive success.

Poster 28 - **Discovering the genetic architecture of pancreatic cancer through whole genome sequencing,**

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Pancreatic ductal adenocarcinoma (PDAC) is projected to become the second cause of cancer-related deaths by 2030. The germline susceptibility loci of PDAC consists of around 40 single nucleotide variants. However, these SNVs explain around 5% of the heritability, thus more need to be identified. Additionally, germline genetics could have a role in PDAC treatment since BRCA1/2 germline mutation carriers have a better prognosis, these mutations are present in only 5% of PDAC patients, suggesting that additional mutations play a key role. To identify novel variants associated with PDAC risk a whole genome (WGS) on 1500 PDAC cases and 1500 controls was carried out. The samples belonged to the the PANcreatic Disease ReseArch (PANDoRA) consortium. The aims of the project were: 1) To identify novel variants associated with PDAC risk. 2) To compute a new polygenic risk score (PGS). 3) To test the variants and the PGS in 600 IPMN patients. 4) To identify variants associated with PDAC prognosis. Investigating the whole genome represents a clear point of innovation compared to all studies conducted so far and is the only possible strategy to identify variants of the genome without a priori knowledge on their function. Unravelling the genetic architecture of the disease will have a significant impact on understanding the aetiology of PDAC and will represent a significant first step into the identification of high-risk individuals that could benefit from targeted screening and surveillance programs. The analysis are currently ongoing at the National Facilities.

Poster 29 - **Determining the Pathogenicity of Genomic Variants of Uncertain Significance in Cardiomyopathies Using CRISPR/Cas9 and Human-Induced Pluripotent Stem Cells**

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Cardiomyopathies are a heterogeneous group of myocardial disorders characterized by structural and functional abnormalities, classified into hypertrophic, restrictive, dilated (DCM), and arrhythmogenic (ACM) forms. Their etiologies include acquired causes and genetic mutations, which account for approximately 30–50% of cases. Clinically, patients may present with heart failure symptoms and arrhythmias, with an increased risk of sudden cardiac death. Genetic factors play a key role in diagnosis, risk stratification, and family screening, and may directly influence clinical management, including preventive implantation of cardiac devices.

Advances in next-generation sequencing have expanded the understanding of cardiomyopathy genetics, revealing both monogenic and polygenic contributions and significant genetic and phenotypic overlap among different subtypes. In particular, DCM and ACM share important clinical and molecular features, making genetic investigation essential for accurate risk assessment and sudden cardiac death prevention. However, a major challenge in clinical genetics is the high prevalence of variants of uncertain significance (VUS), which complicates result interpretation and clinical decision-making.

This project aims to establish a reproducible in vitro approach to evaluate the pathogenicity of VUS, focusing on desmosomal and nuclear gene variants (PKP2, DES, DSP, DSG, RBM20, LMNA, and FLNC) associated with DCM and ACM. Three pairs of patients will be selected, each including one individual carrying a pathogenic/likely pathogenic (P/LP) variant and another carrying a VUS in the same gene. Patient-derived iPSC lines will be generated, differentiated into cardiomyocytes, and assessed through functional cellular assays. Electrophysiological properties will be analyzed using patch-clamp recordings, while mechanical performance will be evaluated using the IonOptix contractility system. Subsequently, one variant pair showing functional alterations will be further investigated using CRISPR/Cas9-mediated gene editing to create isogenic models, enabling definitive genotype-phenotype correlations and reducing reliance on patient-derived cells. This workflow is expected to improve VUS interpretation and support personalized management in DCM and ACM.

Session 2

Poster 1 - miR-17~92 cluster members modulation mitigates oxidative stress in sodium iodate-induced ARPE-19 AMD model

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Age-related macular degeneration (AMD) is a leading cause of blindness, characterized by oxidative stress and mitochondrial dysfunction in the retinal pigment epithelium (RPE). This study investigates the therapeutic potential of some members miR-17~92 cluster in mitigating cellular damage induced by sodium iodate (NaIO₃), a potent oxidant used to mimic the pathophysiology of dry AMD. Since members of the miR-17~92 cluster are part of the Oxygen Responsive miRNAs (OxymiRs), which are differentially expressed in marine animals during hypoxia/anoxia conditions, and are conserved in human, we hypothesized that perturbing this miRNA cluster could modulate oxidative stress response and mitochondrial dynamics in ARPE-19 cells. Preliminary data suggested a pro-survival effect of miR-17/20a/92 inhibition in NaIO₃ ARPE-19 cells. We employed a high-throughput transcriptomic approach using ARPE-19 cells. Cells were subjected to NaIO₃ induced stress following overexpression and inhibition of members of the miR-17~92 cluster alone or in combination (miR-17/20a/92). RNA sequencing was performed to capture the global transcriptional landscape, followed by bioinformatic integration of differential gene expression, pathway enrichment analysis, and correlation with a miR-CLIP assay (targetome). The inhibition of miR-17~92 cluster members significantly alter the expression of genes involved in the HIF1A stress response and mitochondrial dynamics pathways (e.g., mitophagy, ferroptosis). By correlating the transcriptome data with the targetome data, we could have a clear picture of which differentially expressed transcript was a real direct/indirect target of the miRNAs (e.g., HIF1AN). This project advances the understanding of the miRNA-mediated regulation in eye disease context. By utilizing high-throughput transcriptomics, we move beyond single-gene studies to a systems-biology perspective, mapping the complex regulatory networks governed by miRNAs. The findings provide a framework for developing miRNA-based therapeutics that can simultaneously address multiple pathological hallmarks of AMD, where current “monotherapies” often are not effective.

Poster 2 – Mechanistic insights into AFFND1-associated genetic variants through human iPSC-derived organoids

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Acrofrontofacionasal Dysostosis type 1 (AFFND1) is an autosomal recessive disease with an estimated prevalence of <1:1,000,000 individuals and only four families described in literature. Patients display facial and skeletal abnormalities, short stature, and intellectual disability. We previously reported the identification of a novel homozygous truncating mutation (c.6237-8C>G) in the NBAS gene in the Indian patients and a homozygous intronic variant in the PIGB gene (c.795-19T>G) in the Brazilian ones. For both variants we showed a detrimental effect on splicing, but we could not provide final demonstration of the causative role with respect to disease pathogenesis.

In this project we aimed to move from variant association to causality by generating in vitro AFFND1 disease models. Namely, we proposed to generate human iPSCs carrying either the c.6237-8C>G NBAS or the c.795-19T>G PIGB genetic variant; to perform a complete phenotypic and functional characterization of the gene-edited iPSCs; and to develop 2D/3D bone organoids for in-depth and dynamic assessment of bone development in the presence of the NBAS or the PIGB genetic variant. The significance of our project is the possibility to advance knowledge of basic mechanisms of skeletal developmental diseases and to establish a useful technological platform to model other skeletal developmental disorders.

Gene-edited iPSCs carrying the PIGB c.795-19T>G variant at the homozygous state were generated by the GEDI Facility, demonstrating that the PIGB gene (and specifically the genomic region of interest) is efficiently targetable through CRISPR-mediated homology directed repair; however, the strategy applied prevented aberrant splicing. Conversely, the NBAS gene editing was challenging and no biallelic edited clone was obtained despite applying diverse strategies. These failures could not have been anticipated a priori, nonetheless, the experience gained informed a different editing strategy which should be successful, and we deem the relevance of the expected outcome supports further attempts.

Poster 3 – **Structural studies on the epigenetic mechanisms of Wnt gene transcription activation and transcriptional control**

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The spatiotemporal coordination and the fidelity of execution of transcriptional programs are key processes for development and tissue homeostasis. Consistently, reprogramming and unlocking of phenotypic plasticity have been recently recognized as hallmarks of cancer. In vertebrates, transcription activation is promoted by histone acetylation, deposited at localized genomic sites by the acetyltransferases p300/CBP, which associate to transcription factor-containing macromolecular complexes. Specifically, p300 acts as a coactivator of Wnt gene transcription by binding to the β -catenin subunit of the Wnt enhanceosome. Through proteomic experiments conducted at the SB-NP of Human Technopole, we identified a new layer of p300-mediated Wnt gene transcription regulation involving components of the INHAT complex and the chromatin-associated protein NuMA. Reconstitution experiments confirmed the direct interaction between the proteins of this circuitry that we are characterizing structurally and functionally. Additionally, to investigate how mechanisms of transcription termination and their dysregulation impact on cancer cell viability, we characterized structurally the architecture and the RNA-binding properties of the 3'-end pre-mRNA processing complex CStF. Mass-Spec Cross-linking experiments coupled to RNA-binding assays and Cryo-EM single particle analyses uncovered the organizational principles and the RNA-recognition mode of CStF, and guided the design of transcriptional experiments in cells. Collectively, our studies are elucidating new layers of Wnt-gene transcriptional epigenetic control and mRNA processing in eukaryotes, which will eventually pave the way to the identification of novel actionable targets for specific pharmacological treatment of Wnt-addicted and transcriptionally-altered cancers.

Poster 4 - **Monitoring immunesurveillance: Analysis of repetitive Elements and Fragmentomics in Tissue and Blood**

Giovanni Crisafulli*^{1,2}, Emma Tassanelli¹, Alessia Anastasia¹, Paolo Battuello², Alberto Bardelli²

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Immunotherapy is transforming cancer treatment across multiple tumor types and clinical settings. However, the absence of reliable biomarkers for predicting immunotherapy response remains a significant challenge. Current approaches, including PD-L1 immunohistochemistry and tumor mutational burden, are limited by technical constraints such as tissue availability and intratumoral heterogeneity. Moreover, genomic strategies aimed at understanding how the immune system is modulated during therapy remain insufficiently explored.

In this context, dysregulated repetitive sequences and transposable elements (TEs), which constitute nearly half of the human genome, have emerged as a promising area of investigation. Aberrant TE expression, often driven by cancer-associated epigenetic alterations, has been associated with immune activation and neoantigen production. These observations underscore the need to elucidate the biological processes underlying TE-driven immune modulation in order to develop biomarkers that accurately reflect tumor-immune dynamics.

In parallel, we have pioneered molecular analyses of circulating cell-free DNA (cfDNA) through liquid biopsy approaches. Liquid biopsies provide a minimally invasive alternative to tissue-based assays, overcoming limitations related to tumor heterogeneity and sample accessibility. Recent advances in understanding DNA fragmentation during cell death (fragmentomics) have expanded the biological insights obtainable from cfDNA, demonstrating its potential to reveal transcriptionally mediated biological processes and enabling innovative strategies for real-time monitoring of immune activation.

To maximize the effectiveness of immunotherapy, it is essential to decode the mechanisms that initiate and sustain immune responses against tumors. A deeper understanding of these processes could enable the identification of novel biomarkers capable of capturing the complexity of tumor-immune interactions longitudinally during treatment, thereby improving clinical decision-making.

Nanopore sequencing technology is particularly well suited to explore these opportunities. This project aims to conduct a comprehensive parallel molecular analysis of tissue and blood samples from preclinical models (WP1-2) and colorectal cancer patients with clinically annotated responses to immune therapies (WP3). This integrated approach will enhance our understanding of the mechanisms driving immune activation and support the development of a novel blood-based methodology to monitor immune system engagement in a clinical setting.

Poster 5 – **INNOVATIVE APPROACHES TO QUANTIFY AN ESTABLISHED BIOMARKER: AI APPLIED TO PHOSPHORYLATED-ALPHA-SYNUCLEIN IN SKIN BIOPSY**

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Multiple system atrophy (MSA) is a neurodegenerative disorder clinically characterized by various combinations of parkinsonian, cerebellar, autonomic, and pyramidal signs (1). Differentiating MSA from Parkinson's disease (PD), particularly in the early phase, may be challenging. These diseases share a common pathological mechanism that involves alpha-synuclein aggregation both in the central and peripheral nervous system (2). As for the peripheral nervous system, skin biopsy has emerged as a diagnostic tool allowing to identify phosphorylated alpha-synuclein in skin nerves (3).

We defined an automated digital pathology workflow to quantify phosphorylated alpha-synuclein within PGP 9.5-positive fibers in skin biopsies obtained from cervical and distal leg sites in order to distinguish MSA (n=40) and PD (n=30). In detail, skin biopsies were subjected to automated immunofluorescence staining on a Leica Bond RX, high-resolution whole-slide-images were then acquired on the Zeiss Axioscan Z.1 at Human Technopole, and we took advantage of artificial intelligence- (AI)-assisted analysis. We also applied super-resolution confocal microscopy to deeper investigate specific aspects revealed by the Axioscan acquisition.

We identified phosphorylated alpha-synuclein within PGP 9.5 positive fibers mainly in somatic skin nerves in MSA, whereas in PD it was predominantly detected in autonomic fibers, at both cervical and distal leg sites, as previously described (4). Furthermore, PGP 9.5 nerve fibers were absent or altered mainly in the autonomic structures, only at distal leg site in MSA patients. All together, these aspects well-correlated with clinical phenotypes and are crucial for distinguishing MSA and PD patients. We obtained a sensitivity of 81% for PD and 76% for MSA and a specificity of 96% for control subjects.

These results move towards personalised medicine in PD and MSA, being essential not only for an early diagnostic purpose, but also as a quantitative biomarker for monitoring future therapeutic approaches.

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2. Spires-Jones TL et al. Acta Neuropathol. 2017;134(2):187-205.

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4. Donadio V et al. Sci Rep. 2018 Sep 24;8(1):14246.

Poster 6 – **Cell type-specific vulnerability to the toxic effects of Tau in animal models of FTD**

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Frontotemporal dementia (FTD) is the second most common cause of early-onset dementia after Alzheimer's disease and remains incurable. Tau-associated FTD is driven by toxic Tau aggregation that disrupts mitochondrial function, leading to neurodegeneration and progressive cognitive decline. A key unresolved challenge is understanding how Tau toxicity differentially affects brain cell types and whether endogenous protective pathways can be therapeutically leveraged.

This project introduces a novel disease-modifier framework by interrogating SUMOylation—a post-translational modification that our laboratory has identified as selectively impaired in FTD—as a regulator of Tau-induced mitochondrial dysfunction. In contrast to prior studies focused on SUMO1, our work demonstrates that SUMO2 exerts a protective function, reducing Tau aggregation, restoring mitochondrial homeostasis, and improving cognitive performance in animal models of Tau-FTD. These findings represent an original conceptual shift in how SUMO paralogs are understood in tauopathies.

Access to the National Facility (NF) for Genomics is essential to the innovation and feasibility of this project. NF-enabled resources allow us to integrate single-nucleus RNA sequencing generated in our laboratory with high-resolution spatial transcriptomics performed at Human Technopole. This complementary, multi-modal approach enables cell-type- and region-specific mapping of Tau-driven transcriptional and mitochondrial stress pathways, as well as their restoration by SUMO2 conjugation—analyses not achievable without NF access.

Scientifically, this work will advance the field by defining SUMO2-dependent protective programs across neuronal and non-neuronal populations, adding a new mechanistic dimension to Tau biology and mitochondrial dysfunction in FTD. More broadly, the insights gained are expected to have cross-disease relevance for Alzheimer's and Parkinson's diseases, where Tau pathology, SUMOylation imbalance, and mitochondrial dysfunction converge. Importantly, NF access has expanded the PI's research capabilities and methodological expertise, positioning this project as a foundation for sustained innovation at the interface of functional genomics, neurodegeneration, and therapeutic discovery.

Acknowledgments: We thank Dario Ricca (NF for Light Imaging); Luca Rotta, Carola Maria Conca Dioguardi, and Clelia Peano (NF for Genomics).

Poster 7 – High-resolution cell sorting of bovine spermatids and epididymal spermatozoa at different stages of maturation

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Sperm development relies on spermatogenesis, a highly dynamic process characterized by dynamic changes in DNA content and associated epigenetic modifications, in chromatin organization, and cellular morphology. Dissecting the molecular mechanisms underlying sperm maturation remains challenging due to the coexistence of heterogeneous germ cell populations within the testis and along the epididymis, which cannot be efficiently resolved using conventional flow cytometry approaches in cattle.

Through access to the IU3 National Facility for Light Imaging, we implemented innovative imaging strategies based on the Cytex ImageStream MKII flow cytometer and BD FACS Discover™ S8 spectral and imaging cell sorter, enabling the integration of fluorescence intensity, cell size, granularity, and morphology-derived features. This approach allowed the visualization and identification of the different possible population to sort with the MKII, first, and then the resolution and isolation with the Discover S8 of spermatid stages that are inaccessible to standard cell-sorting technologies.

Because the testis and epididymis contain highly heterogeneous germ-cell populations, dissecting sperm-maturation mechanisms remains difficult with conventional flow cytometry in cattle. At the IU3 National Facility for Light Imaging, we employed the BD FACS Discover™ S8 spectral and imaging cell sorter together with the Cytex ImageStream MKII to develop an imaging-integrated cytometry workflow. The Discover S8 enabled resolution through morphological imaging discrimination and fluorescence sufficient to identify and sort previously inaccessible spermatid stages. The ImageStream MKII provided true image data at 60X magnification that helped to refine our interpretation of the morphological features associated with the Discover S8-defined stages. Testicular and epididymal samples (caput, corpus, and cauda) were obtained from sexually mature Piemontese bulls and subjected to purification prior to analysis. Using SYTO16 staining combined with physical and imaging-based parameters, we successfully separated two distinct spermatid populations corresponding to early round spermatids (steps 1-9) and late elongated spermatids (steps 13-16), as confirmed by stage-specific nuclear morphology under epifluorescence microscopy. Intermediate stages showed lower separation efficiency, consistent with ongoing nuclear remodelling. In parallel, epididymal spermatozoa were resolved into two reproducible populations across all epididymal regions, characterized by high or low PNA-Alexa Fluor™ 488 fluorescence intensity, reflecting acrosomal membrane rearrangements associated with sperm maturation. Ongoing functional studies aim to elucidate the biological significance of these populations.

Sorted cell populations yielded sufficient material for downstream molecular analyses, including RNA-seq and Reduced Representation Bisulfite Sequencing RRBS, which are currently underway. Access to the National Facility was instrumental in enabling this work, providing

advanced instrumentation and expertise that significantly expanded the scope and impact of the Principal Investigator's research. Beyond its relevance to cattle reproduction and genetic improvement, this study establishes a translational framework with potential implications for human reproductive biology, offering new opportunities to investigate the cellular and molecular determinants of male fertility, diagnose spermatogenic defects, and ultimately inform the development of improved assisted reproductive technologies. Collectively, this work supports efforts to enhance genetic gain and promote long-term sustainability in cattle breeding programs, while also contributing to broader advances in reproductive health.

Poster 8 – Spatial transcriptomic landscape of ischemic stroke clots

Stefano Fumagalli, Simone Bellavia, Tizibt Ashine Bogale, NF-GENOMICS

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Ischemic stroke is determined by cerebral blood vessel occlusion, due to cardioembolic or atherosclerotic causes. Intravenous thrombolysis is the primary medical treatment, but it achieves recanalization in only a third of patients. Clot resistance to thrombolysis is influenced by its cellular and molecular characteristics, which may vary upon stroke etiology. Preliminary data of ours identified different inflammatory mechanisms of cardioembolic vs. atherosclerotic strokes, namely that the latter are linked to increased activation of the complement system. As a consequence, it is likely that the resulting clots have specific composition and may respond differently to thrombolysis. This project aims at deeply analyzing clot retrieved from stroke patients integrating spatial transcriptomic with structural analysis to advance our understanding of stroke pathophysiology. We collected 24 formalin-fixed and paraffin-embedded thrombi, equally split into cardioembolic, atherosclerotic or of undetermined stroke causes. Their analysis was done through the access to the National Facility of Genomics. The clots, which are rich in cell debris, lipids, a-nucleated cells like platelets and red blood cells, fibrin and collagen, posed critical challenges for obtaining a reliable spatial transcriptomic profile. Based on histological analysis, we therefore selected a few clots with dense cellularity and appropriate RNA content, which could be sequenced. The data are now under processing. Shall the experiment provide data of enough quality, we will be able to provide high-resolution localization of gene expression profiles while retaining the structural information of the thrombus. This would be an innovative result, allowing to overcome the limits of previous studies, focused selectively on morphological or molecular information.

Poster 9 – **Systemic maintenance with Cyclophosphamide Associated or not to Vinorelbine after induction chemotherapy in metastatic Pancreatic Ductal Adenocarcinoma (PDAC) to Enhance tumor Growth Eradication (SCAVENGER - PACT-35 trial): rationale and clinical trial design**

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Pancreatic ductal adenocarcinoma (PDAC) is a malignancy with dismal prognosis and limited therapeutics options, thus representing an unmet need in clinical oncology. At 6 months 45-75% of metastatic patients (pts) experience progression-free survival (PFS-6) by current chemotherapies. One of the mechanisms involved is the reactivation of effector CD8 T-cells and the downregulation of the immune-suppressive PDAC milieu. However, within 6 months of chemotherapy completion, less than 4% of pts remain progression-free, suggesting a quick loss of the chemo-induced benefit. In these light, new strategies for enhancement of response duration, by using both de-potentiated chemotherapy or selected target agents, have been explored with encouraging results. Our group recently proved the benefit of metronomic cyclophosphamide (CTX) as maintenance therapy in advanced PDAC pts, showing a maintenance PFS-6 of 26.2%. Available preclinical data showed that CTX may induce an immune system re-modulation, increasing the number of CD4 and CD8 T cells and lowering Treg cells, with a synergistic effect when associated to vinorelbine (VNR). We tested for the first time a cohort of metastatic PFS-6 PDAC pts, with maintenance metronomic treatments as follows i) CTX ii) CTX plus VNR iii) observation. Our aim is to verify the benefit of maintenance therapies, focusing on their immunological modulation ability by analyzing the circulating immune cells phenotype by flow-cytometry approaches.

Poster 10 – **Harnessing Long-Read Sequencing for Unveiling Structural Variants in Pancreatic Cancer: A Step Toward Precision Medicine.**

Manuel Gentiluomo* (1), Riccardo Farinella (1), Chiara Corradi (1), Daniele Campa (1)

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Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, with limited opportunities to personalize adjuvant chemotherapy despite substantial inter-individual variability in treatment response. Although germline genetic variation is increasingly recognized as a contributor to cancer susceptibility and therapeutic outcomes, current clinical implementation relies almost exclusively on single-nucleotide variants, leaving the role of structural variants (SVs) largely unexplored. This knowledge gap is mainly driven by the intrinsic limitations of short-read sequencing technologies in resolving complex genomic rearrangements.

This 24-PILOT project leverages access to the Human Technopole National Facility for Genomics to apply whole-genome long-read sequencing to a cohort of resected PDAC patients treated with adjuvant chemotherapy. Using Oxford Nanopore technology, the project will enable accurate, genome-wide detection of germline SVs—including deletions, duplications, insertions, and complex rearrangements—that are typically inaccessible to standard sequencing approaches.

The study is expected to deliver three main outcomes:

- (i) the generation of a high-quality catalogue of germline SVs in PDAC patients;
- (ii) the identification of SVs potentially associated with chemotherapy response, overall survival, and disease-free survival through integration with detailed clinical follow-up data;
- (iii) the establishment of an analytical framework for incorporating long-read SV data into pharmacogenetic studies of PDAC.

By providing access to advanced sequencing infrastructure and specialized technical support, the National Facilities have been essential in enabling this investigation and in overcoming methodological barriers that would otherwise preclude such analyses. Beyond its immediate scientific objectives, this project is expected to produce robust pilot data to support future large-scale validation studies, strengthen the Principal Investigator's expertise in long-read genomics, and foster new collaborations within the National Facilities user community, ultimately contributing to the development of more personalized therapeutic strategies for PDAC patients.

Poster 11 - Structural pharmacology of chronic pain: cryo-EM of HCN2 channel

Andrea Saponaro

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Hyperpolarization-activated Cyclic Nucleotide-gated 2 (HCN2) channel and more precisely its sensitivity to cAMP, is emerging as a promising pharmaceutical target for pathological pain. Classical analgesic opioids, which indirectly affect HCN2 by inhibiting the cAMP pathway, have severe long term side effects due to cellular remodeling. By focusing on HCN2 the project proposes alternative pharmaceutical strategies with reduced toxicity compared to opioids. The only available drug for HCN channels, Ivabradine (Saponaro et al., PNAS 2024), is not useful for pain management since it causes bradycardia due to inhibition of HCN4, the heart isotype. To overcome this limit, a structural comparison of the three main HCN isotypes (1, 2 and 4) could lead to the identification of the molecular bases for the HCN2 specific sensitivity of cAMP. This structural approach has been successfully confirmed by the comparison between the structure of HCN4, the cardiac isotype, and the one of HCN1, the principal brain isotype. Indeed, local differences observed in the connection between the cytosolic and the transmembrane region of HCN4, compared to HCN1, translate into functional differences (Saponaro et al., Mol Cell 2021). In this light, the project aims to describe, in atomic details, the mechanism of cAMP regulation of the full-length HCN2 channel by using single particle cryo-EM. This will pose the necessary basis for the identification of isotype-specific pockets that can become drug targets.

Poster 12 - **Comprehensive Genomic Analysis of Undiagnosed Muscular Disorders: From Novel Variants to Disease Mechanisms**

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Primary muscle disorders are clinically and genetically heterogeneous. The achievement of a reliable molecular diagnosis can improve clinical management, provides appropriate genetic counseling and enables potential therapeutic options. In the last 6 years, 285 patients with the suspect of primary muscle disease underwent NGS-based molecular testing in our Center. Overall diagnostic yield was 59%. Disease-causing variants were identified in 110 patients (38%: definitive diagnosis). Likely pathogenic variants and/or variants of uncertain significance were found in 59 additional probands (21%: probable diagnosis). Whole exome sequencing contributed to identify 3 novel disease genes (GUK1, POPDC2, TNNT1).

Although successful, this approach is blind to complex structural variants, repeat expansions, quantitative and intronic changes that affect gene regulation.

To overcome these limitations, in this project we apply genomic technology to well-characterized undiagnosed patients presenting neuromuscular disorders.

112 DNA samples passed initial quality check (concentration and integrity). 100 samples representing 60 independent undiagnosed probands and available (affected or unaffected) family members were selected for Whole Genome Sequencing analysis (provided by HT National Facility for Genomics). Cases are stratified by phenotypic clusters: muscular dystrophies (n=12), congenital myopathies n=22), metabolic myopathies (n=18), other rare neuromuscular disorders (n=8) and show biochemical or histopathological (muscle biopsy) evidence of disease. Libraries have been successfully generated and run.

Bioinformatic analysis and variant prioritization is currently ongoing. We are analyzing trio samples (proband and parents) or multiple affected subjects from the same pedigree, to enhance the ability to identify de novo or (shared) inherited defects and determine variant inheritance patterns.

We aim to increase the diagnostic rate in our cohort of patients and to unravel new disease genes in primary muscle disorders by implementing first class genomic analysis with in-house set of tools for molecular, biochemical and functional validation of candidate variants.

Poster 13 - The PACECOR project: Pre-targeting ACE2-binding of CORonavirus

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The "Pre-targeting ACE2-binding of CORonavirus" (PACECOR) project is a comprehensive effort to develop a pharmacological intervention for treating coronavirus-infected individuals or those in the early stages of a pandemic to prevent disease spread. We have produced and validated an innovative bimodal antiviral complex called PACECOR. The pharmacological probe is designed to exploit the targeting efficiency of a synthetic, next-generation antagonist of angiotensin converting enzyme 2 (ACE2), which is functionalised with a biotin moiety to enable an almost irreversible binding with an engineered avidin anchor of a second competitor in the Spike protein-ACE2 interaction. Our in vitro evidence shows that: the three components can form a complex; once the complex is assembled the affinity of the molecules is boosted to 49pM assessed by SPR; pretreatment of susceptible cells to SARS-CoV-2 infection with the 2 molecules protect cells from viral infection, as by the design of this approach. In vivo experiments are ongoing to define bioavailability of the molecules in vivo on mice expressing human ACE2 and in the future to assess protection from SARS-CoV-2 infection. Understanding of the composition of the complex and the epitopes bound among the components of the ACE2/ DX600-biotin / RBD-monodin ternary complex will be crucial to design optimized versions of the molecules and instrumental to get insights into the definition of their mechanism of protection and for publication of those results. The aim of the proposal was to investigate the PACECOR complex structure formed by the extracellular moiety of human ACE2, the RBD-monodin protein chimera, and the DX600-biotin small molecule to gain an understanding of the mechanism of action and ultimately develop improvements to the PACECOR antiviral strategy through Cryo-EM screening at the National Facility for structural biology of the Human Technopole.

Poster 14 - **Genomic insights into the spread of the alien and invasive mosquito *Aedes koreicus***

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Aedes koreicus, an invasive mosquito native to northeastern Asia, can act as a vector of arboviruses and nematodes. It has rapidly expanded across Europe since 2008, but the origins, routes and drivers of this invasion remain unresolved, limiting evidence-based strategies for surveillance and vector control. Here we generate the first whole-genome dataset for this species, sequencing 192 specimens spanning its native distribution and invaded European regions. This unprecedented numerical and geographic coverage represents a breakthrough compared to previous studies based on limited sample sizes and low-resolution markers. Preliminary analyses on the first 96 individuals yielded promising genomic resources: despite heterogeneous sequencing yield (6.9–129.3 Mb) and coverage (3×–33×), >90% of reads mapped to the reference genome, and stringent variant calling produced 14,355,191 high-quality biallelic SNPs. This panel, once implemented with analyses from the second batch of samples, will enable robust inference of population structure, admixture and introduction routes in *Ae. koreicus*. Access to the NovaSeq 6000 platform through National Facilities' call was essential to obtain robust and affordable whole-genome sequencing for a species whose genomics remain scarce. Beyond population genomic inferences, data obtained from this project represent a breakthrough in genomic resources for a previously understudied invasive species.

Poster 15 - **DETECTIVE: Defining the genomic landscape of metabolic steatotic liver disease**

Valenti L, Malvestiti F

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Metabolic dysfunction-associated steatotic liver disease (MASLD), driven by insulin resistance and affecting one-third of adults, is the leading cause of liver disease. Risk stratification biomarkers and effective treatments remain unmet needs. Common genetic variants linked to hepatic fat retention explain only a fraction of the large MASLD heritability.

At the Fondazione Biobank, we confirmed known MASLD risk loci and highlighted rare germline variants contribution in cases with advanced MASLD and controls.

The present study aims to uncover the full genomic landscape of MASLD in an Italian population, focusing also on rare, structural variants, and on clonal hematopoiesis. Using whole-genome sequencing (WGS), we will analyze germline and somatic variants linked to advanced MASLD, identifying those increasing the risk of liver-related outcomes. To this end, we will sequence peripheral blood DNA samples 800 people with advanced MASLD and 2,000 matched controls with extensive characterisation and consider also >1,000 healthy individuals. Liver tissues will also be sequenced in a subset. Genetic associations will be tested using genome-wide regression models.

Findings are expected to reveal key genetic determinants of advanced MASLD, novel rare variants, and to improve risk stratification through polygenic risk scores, aiding disease subphenotypes identification and therapeutic target discovery.

Poster 16 – Characterization of the NF1 role in microtubule dynamics through Cryo-EM and XL-MS: rediscovering a tumor suppressor

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Neurofibromin 1 (NF1) is a tumour suppressor, originally identified as the gene responsible for neurofibromatosis type 1 disease, but increasingly recognized as a somatic driver of numerous cancers. NF1 protein is well known as a regulator of RAS GTPase activity and this role is extensively covered in literature. NF1 biochemistry has remained understudied for several years due to the difficulties in its manipulation; recently solved structure has revealed important details on its interaction with RAS, but many other interactions suggested over the years remain underexplored.

We have accumulated extensive evidence pointing to a role for NF1 in regulating microtubule (MT) dynamics through a RAS-independent mechanism. In particular, using CRISPR-engineered models of breast cancer and biochemical assays on purified recombinant NF1, we demonstrated that NF1 physically interacts with microtubules and regulates their dynamics both in vitro and in vivo. In particular, NF1 appears to protect cells from MT damage induced by a specific class of microtubule-depolymerizing drugs, maytansinoids, which are currently routinely used as payloads in approved-antibody-drug conjugates.

Our results shed new light on the molecular basis of neurofibromatosis and NF1-associated cancers, suggesting that defective microtubule damage repair may be involved, and providing a relevant biomarker to guide patient treatment.

To further elucidate the molecular mechanism of action and to define how NF1 interacts with MTs, structural insights into the NF1-MT complex are required. To this end, we accessed the National Facilities through the 24-Pilot Calls program, where we obtained initial cryo-electron microscopy (Cryo-EM) and crosslinking mass spectrometry (XL-MS) data. Notably, XL-MS data enabled the identification of at least one interaction interface between MTs and the N-terminal region of NF1. By mapping the cross-links onto available 3D structures of NF1 and MT, we first confirmed that the binding site on tubulin is located on the outer surface of the microtubule rather than on its inner cavity, in agreement with the mechanism of action of NF1 that we recently discovered. These findings will be further validated through the generation and functional characterization of N-terminal NF1 mutants, which is currently ongoing.

Overall, access to the National Facilities enabled the acquisition of initial yet essential results for the development of structural models of the NF1-microtubule interaction.

Poster 17 - **Integrative multi-omics analysis of treatment resistance and disease progression in chronic lymphocytic leukemia**

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Chronic lymphocytic leukemia (CLL) is the most frequently diagnosed form of leukemia in Western populations. Despite recent therapeutic advances, CLL remains incurable. The disease course is highly variable: while some patients may never require intervention, others face disease progression and a poor outcome, implying great evolutionary diversity across patients. Current risk assessment methods are insufficient to reliably predict who will eventually require treatment, preventing timely intervention. To improve patient risk stratification and guide therapeutic decisions, we aimed to define the molecular determinants of disease progression from an indolent, pre-leukemic phase to overt CLL in need of treatment. Within the 24-G-PILOT call at the National Facility For Genomics, multiome RNA+ATAC single-cell sequencing of 8 “watch-and-wait” CLL patients (i.e., treatment free) versus 8 “pre-treatment” cases (i.e., patients in need of therapy, sampled before treatment start) was performed. Gene expression and chromatin organization analyses identified several B-cell clusters in individual patients, pointing to significant intra-leukemia phenotypic diversity. Preliminary analysis of the biological processes characterizing the different clinical groups suggests that in ‘high-risk’ IGHV-unmutated CLL, patients not requiring treatment (indolent disease) display a functionally restrained BCR program with dominant negative feedback, checkpoint activation, and reduced migratory capacity, compared with patients that started therapy; these features are consistent with a restrained, non-proliferative cellular state, and may explain why some unmutated CLL patients behave indolently despite carrying a high-risk IGHV genotype. On the other hand, in ‘low-risk’ IGHV-mutated CLL, patients requiring therapy are characterized by loss of cellular quiescence and reactivation of metabolic and NF- κ B programs, as well as BCR signaling, enabling re-entry into a proliferative state that may explain disease progression despite a favorable IGHV genotype. Chromatin accessibility data confirmed these gene expression changes. Integrated multimodal analysis at the cell cluster level is underway, to identify subpopulations that characterize each patient, and link this information to the clinical course.

Poster 18 – **Spatial Multi-omics Analysis of Neural Stem Cell-Based Therapy in ALS/FTD Chimeric Organoids: Decoding Disease-Modifying Mechanisms and Non-Cell Autonomous Death**

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative disorders with no effective therapies. Although neural stem/precursor cell (NSC/NPC) transplantation has shown therapeutic promise, its mechanisms of action remain poorly defined, limiting clinical translation. Progress has been constrained by reliance on fetal-derived cells, limited scalability, ethical concerns, and animal models that fail to capture human-specific disease mechanisms. We present a novel chimeric human brain organoid grafting platform to dissect and optimize NPC-based therapies in a human-relevant context. Cortical brain organoids derived from induced pluripotent stem cells (iPSCs) carrying ALS/FTD-associated mutations (C9ORF72, TDP43, and SOD1) recapitulate key pathological hallmarks, including protein and RNA aggregation, neuronal dysfunction, and non-cell-autonomous neurodegeneration. Transplantation of healthy NPCs into diseased organoids enables direct investigation of donor-host interactions and therapeutic mechanisms within complex human neural tissue. To define the molecular and cellular basis of therapeutic effects, we integrate spatial and multi-omics approaches, including 10x Genomics Visium spatial transcriptomics, single-cell RNA sequencing, and bulk ATAC-seq of grafted cells. This framework enables spatially resolved mapping of transcriptional and epigenetic changes induced by NPC transplantation across distinct genetic backgrounds and time points. Computational analysis of ligand-receptor interactions and cell-cell communication networks will identify conserved and mutation-specific therapeutic pathways. This study is expected to (i) uncover key molecular mechanisms mediating the beneficial effects of NPC transplantation, (ii) reveal how healthy donor cells remodel the neurodegenerative microenvironment, and (iii) identify predictive markers of therapeutic response. Overall, this work establishes a transformative human platform to guide the rational optimization of cell-based therapies for ALS/FTD and potentially other neurodegenerative diseases.

Poster 19 - **BioSUD prime: A whole genome biobank for the study of Substance Use Disorders**

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Substance Use Disorders (SUDs) are complex conditions shaped by genetic susceptibility and environmental exposures. Although twin studies and GWAS support a heritable component for SUD-related traits, most genomic discoveries have been generated in Northern European or African American cohorts, limiting transferability and potentially missing variants relevant in other ancestries. Southern Italy is an especially informative—and underrepresented—context because its relatively high genomic diversity reflects long-term gene flow from the Balkans, the Middle East, and North Africa.

This project will analyze whole-genome sequences (WGS) from 1,800 individuals from Apulia (Southern Italy), deeply phenotyped through validated questionnaires and clinical/medical reports, to investigate both common and rare genetic contributions to SUD-related behaviours. The originality of the approach lies in combining ancestry-aware whole-genome analyses with integrated psychosocial data to: (i) identify population-specific and shared risk loci; (ii) quantify rare-variant burden in biologically informed gene sets and regulatory regions; and (iii) model gene-environment interactions that may be missed in less diverse or less deeply phenotyped cohorts. We will additionally evaluate the portability of existing GWAS findings and polygenic scores to this Mediterranean population and propose strategies to improve cross-population prediction.

Access to National Facilities enabled the generation of a high-quality, standardized WGS dataset at a scale that would not be achievable locally, accelerating the PI's research timeline and ensuring robust quality control and harmonization. This access strengthens the PI's program by enabling novel analyses of underrepresented European diversity, supporting new collaborations, and establishing a foundational resource for precision public health and addiction genomics in Italy.

Poster 20 - **Building a human iPSC platform for X-linked acrogigantism through the GEDI Facility**

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X-linked acrogigantism (X-LAG) is an ultra-rare and severe endocrine disorder caused by duplications at Xq26 involving GPR101, leading to early-onset pituitary hyperplasia or adenomas with massive growth hormone (GH) hypersecretion and poor response to standard medical therapies. Despite major advances in understanding the genomic and chromatin mechanisms underlying tumoral GPR101 misexpression, a major limitation in the field remains the absence of a human, patient-specific experimental model able to recapitulate hypothalamic-pituitary development and disease pathophysiology.

Through an awarded research service, the GEDI Facility is generating induced pluripotent stem cell (iPSC) lines from peripheral blood mononuclear cells of X-LAG patients and appropriate controls. These iPSCs will constitute a unique resource to establish hypothalamic-pituitary organoids that faithfully model pituitary development and hormone regulation in a patient-specific genetic context. Although reprogramming is currently in progress and experimental data are not yet available, access to the GEDI Facility has been central for my lab research. For instance, the availability of X-LAG iPSC lines has enabled the formulation of a dedicated experimental Aim in our recently awarded project by the Fondazione Cariplo-Telethon funding scheme, aimed at elucidating the role of the Tdark gene OTOS in pituitary hormone secretion and tumorigenesis. X-LAG represents a uniquely informative biological context for this purpose, as OTOS is among the most strongly upregulated genes in X-LAG pituitary lesions and its expression is linked to GPR101 overexpression. By differentiating X-LAG patient-derived iPSCs into hypothalamic-pituitary organoids, we can study the function of OTOS in a genetic and developmental setting that is relevant to the disease, where its regulation is influenced by the native chromatin and transcriptional changes caused by GPR101 duplications.

Overall, this project highlights how important the GEDI Facility has been for generating cutting-edge, human-based models of rare genetic diseases, bridging the gap between genomic discoveries and translational studies.

Poster 21 - Single-Nuclei Multi-Omics Dissection of Transcriptional and Epigenetic Remodeling During Cardiac Aging

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Aging is associated with a progressive decline in myocardial function, increasing susceptibility to heart failure in elderly individuals. This dysfunction is driven by age-dependent transcriptional reprogramming, partially regulated by enhancer activity and leading to metabolic remodelling and impaired contractility. However, the heart is a complex multicellular organ, and how aging affects transcriptional and epigenetic programs across distinct cardiac cell populations and their interactions remains largely unexplored.

In this ongoing study, we are employing an innovative integrative single-cell multi-omics approach to investigate how aging reshapes transcriptional regulation and intercellular communication within the cardiac ecosystem. We are combining single-cell RNA sequencing and chromatin accessibility profiling (snRNA-seq and snATAC-seq) with bulk and cell-type-specific transcriptomic analyses of cardiomyocytes, endothelial cells, fibroblasts, and CD45⁺ immune cells isolated from the left ventricles of young (2 months), adult (6 months), and early-aged (18 months) mice. This approach enables the identification of cell-type-specific enhancer-driven regulatory programs and the reconstruction of signaling networks involved in inflammatory and metabolic remodelling of the aging myocardium, with particular focus on T cell-mediated costimulatory pathways.

Access to the National Genomics Facility at Human Technopole is a key enabler of this project, allowing us to generate single-nuclei multiome ATAC-seq data that would not be accessible within our home institution. This access is substantially enhancing the scope and depth of the Principal Investigator's research by enabling advanced integrative analyses at single-cell resolution and supporting the development of cutting-edge expertise in multi-omics data integration. The results are expected to provide novel mechanistic insights into the epigenetic regulation of cardiac aging and to identify potential therapeutic targets to prevent or delay age-related myocardial dysfunction.

Poster 22 - Eyes on the map: exploring human Uveal Melanoma by Spatial Transcriptomics from primary tumor to liver metastasis to identify new molecular targets involved in tumor-immune contexture

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Uveal melanoma (UM) is a rare and highly aggressive ocular malignancy, with approximately 50% of patients developing metastatic disease, mainly to the liver, for which survival remains poor regardless of treatment. Prognosis is strongly influenced by chromosome 3 monosomy and BAP1 gene inactivation, which associate to altered immune profiles. Clinical results indicate that modulation of antitumor immunity may represent a relevant strategy for disease control. Our laboratory and published data indicate distinct immune regulatory mechanisms involvement in primary and metastatic disease, with activated T cells prevailing in primary UM and suppressive myeloid populations enriched in liver metastases, partially mirrored at the systemic level. A deeper understanding of tumor-immune organization within the immune privileged eye and the immunosuppressive liver environment is therefore required to obtain a comprehensive view of this biologically complex disease at patient level. Through the 2024-PILOT Call of the HT National Facility for Genomics, we obtained access to 10x Genomics Visium HD Spatial Gene Expression platform, for the profiling of four patient-matched primary and metastatic UM FFPE samples from a unique clinical cohort treated at our institute. This proof of principle study demonstrated the feasibility of high-resolution spatial transcriptomics in pigmented tissues. Preliminary analyses identified transcriptionally distinct clusters corresponding to immune, stromal, and specialized epithelial or neuroendocrine compartments, reflecting the biological complexity of UM. Spatial analyses and multi sample integration are ongoing with support from the HT National Facility for Data Handling and Analysis. Overall, this study is expected to provide novel insights into the spatial organization of the UM microenvironment, enabling identification of progression-associated markers with potential implications for precision immunomodulatory strategies in metastatic UM. Access to the HT National Facility enabled analysis by a technology unavailable at the PI's research site, expanding the translational scope of the project and supporting future multicenter studies and grant applications.

Poster 23 – **Upscaling the hCDKL5 recombinant production in an unconventional Antarctic bacterium: a step towards the enzyme replacement therapy for CDD**

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High-accuracy protein structure prediction models have revolutionized protein design by enabling rapid and reliable inference of 3D structures directly from sequence data. In spite of this breakthrough progress, it has not resolved the downstream challenge of producing recombinant proteins in a functional, scalable, and economically sustainable manner. The resulting expansion of the designable protein space has, in fact, exposed the limitations of conventional bacterial, yeast, and mammalian expression systems, which remain inefficient for a significant subset of biologically relevant proteins.

The Antarctic Gram-negative bacterium *Pseudoalteromonas haloplanktis* TAC125 (PhTAC125) has emerged as a promising unconventional production platform. We demonstrated its remarkable versatility as a cell factory for hard-to-express proteins, particularly of human origin, due to its distinctive intracellular physicochemical conditions and protein folding dynamics. These features make PhTAC125 especially suitable for proteins combining structured domains with intrinsically disordered regions, which are notoriously problematic in standard hosts. Human Cyclin-Dependent Kinase-Like 5 (hCDKL5) is a paradigmatic example. It comprises a folded N-terminal catalytic domain linked to a large intrinsically disordered region, a configuration that promotes aggregation and proteolysis in conventional systems. To date, PhTAC125 is the only recombinant platform capable of producing full-length, soluble, and catalytically active hCDKL5. This is particularly relevant because mutations in CDKL5 cause CDKL5 Deficiency Disorder, a severe ultra-rare neurodevelopmental disease lacking curative therapies, for which enzyme replacement therapy shows preclinical promise.

Our team developed a robust laboratory-scale process for the production of the two major cerebral hCDKL5 isoforms with validated *in vitro* activity. Strategic collaboration with the HT Biomass Production Unit enabled the effective transfer, optimization, and scale-up of this process, providing the first experimental validation of the industrial feasibility of the PhTAC125 platform. In parallel, hands-on training of a Ph.D. student ensured efficient knowledge transfer and strengthened the long-term scientific and technological impact of the collaboration.

Poster 24 - **Single-cell transcriptomic profiling of asparaginase response in three cell lines: unveiling novel anticancer mechanisms of an old drug**

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Asparagine and glutamine depletion mediated by L-asparaginase (ASNase) has revolutionized the treatment of Acute Lymphoblastic Leukemia (ALL), yet asparagine dependency and its possible role as a therapeutic target for solid tumors remain underexplored. Previous studies conducted in our laboratories (Pessino 2024, Guardamagna 2023) have proved that ASNase can perturb cell-cycle progression in in vitro solid tumor models, but the exact molecular mechanism underlying such response is still unclear. Here, we used single-cell RNA sequencing (scRNA-seq) to investigate the transcriptional effects of ASNase across hematological and solid tumor models.

scRNA-seq was performed on RS4;11 (ALL), A549 (lung adenocarcinoma), and 786-O (renal clear cell carcinoma), treated with ASNase for 72 hours (0.001 U/ml for RS4;1, or 1.0 U/ml for A549 and 786-O, N=3). Single-cell Gene Expression Flex libraries (10x Genomics) were generated at the Italian National Facility Human Technopole. Sequencing data were processed using Cell Ranger v9.0.0, downstream analyses were conducted in R using Seurat v5.2.1 and DESeq2 v1.46.0 for differential gene expression (DGE), and fgsea v1.32.4 and clusterProfiler v4.14.6 for gene set enrichment analysis based on GO and KEGG databases. ASNase induced a heterogeneous transcriptional response, with 515, 1,877 and 3,025 differentially expressed genes identified in A549, 786-O and RS4;11 cells, respectively. Despite quantitative differences, a transcriptional signature was observed across all models, including upregulation of stress- and ferroptosis-associated genes (e.g., TXNIP, SQSTM1, HMOX1) and downregulation of genes involved in cell-cycle and mitotic processes (e.g., MYBL2, TOP2A, KIF11, CENP family), suggesting activation of stress pathways and repression of proliferative programs.

These results indicate that ASNase elicits a shared transcriptional program never described before across hematological and solid tumor models, supporting its potential repurposing beyond leukemia and providing unique insights in the molecular mechanisms involved in the antiproliferative effect of the drug on solid tumors.

Poster 25 - **Unraveling the role of microglial C1q in neuroinflammation in multiple sclerosis using hiPSC-derived models**

Aletta van den Bosch* (1), Giuliana Mingaj (1), Chiara Dedominicis (1), Edoardo Pedrini (1), Maria Sofia Martire (1), Martina Absinta (1)

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Multiple sclerosis (MS) is a lifelong autoimmune disease of the central nervous system (CNS) leading to disability. Current treatments fail to address CNS-compartmentalized, glia-mediated inflammation, which drives progression independent of relapse activity. Our research using single-nucleus RNAseq revealed that the microglial C1q and C3 play central roles in sustaining smoldering inflammation.

This study aims to dissect the role of C1q in neuroinflammation using advanced in vitro models. Four available hiPSC lines derived from MS patients will undergo CRISPR-Cas9-mediated C1Q knockout (KO) to investigate microglial and glial interactions.

Aim1 focuses on the functional impact of C1Q KO in hiPSC-derived microglia. Assays will measure key functions such as phagocytosis, cytokine production, and transcriptomic changes in response to MS-relevant stimuli (e.g., MS cerebrospinal fluid, myelin debris).

Aim2 examines the effect of C1Q KO microglia engrafted within glia-enriched brain organoids to assess their influence on glial states, neuronal health, and synaptic pruning under inflammatory conditions. Single-cell RNAseq and computational tools will analyze changes in cell-cell communication networks caused by microglial C1Q KO.

We aim to uncover the molecular mechanisms driving microglial complement gene-mediated neuroinflammation and glial crosstalk. The insights gained could inform therapeutic strategies to mitigate neuroinflammation in MS and other neurodegenerative diseases.

Poster 26 - **Exploring human genomic diversity across su-Saharan Africa, the Miffle East, and the Mediterranean Basin to unveil Norther African history**

Giulia Colombo(1), Alessandro Achilli(1), Anna Olivieri(1), Antonio Torroni(1) and Ornella Semino(1)

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Advances in whole genome sequencing have made large scale population studies increasingly accessible, yet modern genomic data remain scarce in key regions such as North and sub Saharan Africa, limiting the reconstruction of demographic and migratory events. This project aims to address these gaps through an integrated analysis of the three genetic systems (autosomal, mitochondrial, and the Y-chromosome) using next generation sequencing technologies on a dataset of 1,000 individuals from Southern Europe, the Middle East, North and sub Saharan Africa. Building on preliminary evidence of four major ancestral components in North African populations (autochthonous, sub Saharan, Middle Eastern and European - Colombo et al., 2025-), comparative analyses with available modern (e.g., the 1000 Genomes Project) and ancient (e.g., Allen Ancient DNA Resource, AADR) databases, will allow us to characterize the genomics of the Mediterranean populations and their genetic links with modern and ancient groups from Europe, the Middle East and sub-Saharan Africa, and the extent to which processes such as mutation, natural selection and admixture have shaped these patterns. Ultimately, the project seeks to generate an integrated framework of global human genetic diversity, with major implications for human genetics and for deeper understanding of the genetic architecture of health-related traits. Moreover, disciplines such as archaeology, linguistics and historiography will also benefit from the results of this project. The massive amount of resources required to satisfy this project could only be fulfilled by the assets made available by the National Facility for Genomics.

Poster 27 - **The mechanism of activation and reaction of MICAL1, the flavoenzyme involved in actin cytoskeleton dynamics.**

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The mechanism of activation and reaction of MICAL1, the flavoenzyme involved in actin cytoskeleton dynamics.

Poster 28 – **Decoding Disease Heterogeneity in Becker Muscular Dystrophy: A Multi-Spatial-Omics Analysis for Therapeutic Target Discovery and Patient Stratification**

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Becker Muscular Dystrophy (BMD) is a severe genetic disorder caused by mutations in the dystrophin gene, resulting in progressive muscle weakness with no available cure. Progressive muscle deterioration involves interactions between inflammation, angiogenesis and fibrosis, representing promising therapeutic targets.

Our innovative spatial multi-omics approach, combines 10X Visium spatial transcriptomics with CosMx spatial molecular imaging, aims to elucidate the intricate interactions among muscle degeneration, inflammation, fibrosis, and angiogenesis with unprecedented resolution. This dual-platform approach enables simultaneous visualization and quantification of molecular signatures within preserved and affected regions of individual BMD muscles, preserving crucial spatial information lost in traditional bulk analyses. By comparing preserved versus affected regions within individual patients and against healthy controls, we aim to uncover protective mechanisms and therapeutic targets.

The project comprises two complementary aims:

In Aim 1, we investigate the interplay among fibrosis, inflammation, and angiogenesis in muscle biopsies from pediatric and adult BMD patients using advanced histological and immunofluorescence techniques. Our preliminary immunofluorescence data reveal age-specific inflammatory and vascular remodeling signatures, suggesting the presence of complex compensatory mechanisms.

Aim 2 focuses on spatial transcriptomic profiling of the fibrosis–inflammation–angiogenesis axis in BMD muscle using the 10X Genomics Visium Spatial Expression platform. The initial cohort of two pediatric and two adult BMD patients has been processed for library preparation and sequencing following quality control assessments for adequate RNA integrity and tissue morphology.

Access to National Facilities enables an integrative approach aimed at identifying novel druggable targets, developing mechanism-based interventions, and discovering non-invasive biomarkers for early diagnosis and disease monitoring. This strategy is particularly relevant for BMD patients, who display striking clinical heterogeneity and currently lack approved therapies.

The expected outcomes will advance personalized interventions and non-invasive biomarkers, ultimately improving disease management and quality of life for individuals with DMD/BMD.

Poster 29 - **Biophysical and biochemical experiments to assess HMGB1-CXCL12-CXCR4 ternary complex**

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The highly dynamic HMGB1-CXCL12 heterocomplex plays a key role in promoting inflammation through activation of the transmembrane receptor CXCR4. We aim to elucidate the structure of the HMGB1-CXCL12-CXCR4 ternary complex, to rationally develop molecules capable of disrupting or inhibiting complex formation, as a novel therapeutic strategy against inflammation-related diseases and cancer.

Through the Structural Biology NF (Biophysics and Structural Proteomics Units) we gained access to cross-linking mass spectrometry (XL-MS), microscale thermophoresis (MST) and bio-layer interferometry (BLI), to investigate the network of interactions characterizing the complex and to measure binding affinities and kinetics of its components.

Extensive trial-and-error experimental sessions, combined with constructive feedback from the NF Biophysics Unit, revealed that our initially purified CXCR4 samples contained a low proportion of properly folded receptor. To address this limitation, we established an optimized protocol involving rapid solubilization of CXCR4 from membrane fragments, followed by immobilization via a biotinylated anti-mCherry antibody on streptavidin-coated BLI biosensors. This strategy maximized preservation of receptor folding during sample preparation and enabled the measurement of nanomolar affinity for the CXCL12-CXCR4 interaction, in agreement with published data.

We performed cross-linking experiments using different stoichiometric ratios of the three complex components and increasing concentrations of sSDA and BS3 cross-linkers. However, MS analysis of the three datasets did not reveal significant inter-protein cross-links, likely reflecting suboptimal component ratios or the need for further enrichment of correctly folded CXCR4.

Although these studies did not yield publishable results, access to the NF instrumentation and the expertise of teams specialized in large protein assemblies and membrane proteins proved invaluable. The hands-on training significantly enhanced our technical skills and deepened our understanding of CXCR4 handling. The successful establishment of BLI assays provides a solid starting point for future structural and inhibitor-screening studies of this medically relevant, yet technically challenging complex.