PhD Day 2024
Wednesday, 16 October 2024
Microsoft Teams: tge4lfx

Program

Session I: Oral Presentation XXXVII Cycle
Chairs: ***Prof. Jessika Bertacchini, Prof. Laura De Rosa, Prof. Alessandra Recchia***

9:00-9:20 Dr. Elisa Adani (tutor Prof. Marigo), "Contribution of metabolic dysregulations to photoreceptor cell death in Retinal Degeneration".
9:20-9:40 Dr. Eleonora Cattin (tutor Prof. Recchia), "CRISPR/Cas9 system to correct laminopathic mutations in patients' cells: generation of in vitro models to identify biomarkers for Emery-Dreifuss Muscular Dystrophy and unravel pathogenic pathways in Hutchinson-Gilford Progeria".
9:40-10:00 Dr. Sara Gentile (tutor Prof. Fanelli), "Insights into GPCR function from network analysis of all structures in the Protein Data Bank".
10:00-10:20 Dr. Matteo Marchionni (tutor Prof. Forcato), "Overcoming Challenges in Cell Population Characterization, From Non-Spatial to Spatial Perspectives in Single-Cell Transcriptomic".
10:20-10:40 Valentina Selleri (tutor Prof. Mattioli, Prof. Bortolotti), "Telemedicine for health status monitoring and correlation with the inflammatory state, with a focus on mtDNA".

Session II: Oral Presentation XXXVIII Cycle
Chairs: Dr. Francesca Chiarini, Prof. Rossella Manfredini, Dr. Ruggiero Norfo

10:40-10:50 Dr. Matteo Bertesi (tutor Prof. Manfredini), "Chromosome 9p duplication enhances hematopoietic stem cells clonogenic potential and promotes immune escape in JAK2-mutant myeloproliferative neoplasms".
10:50 -11:00 Dr. Matteo Corradini (tutor Prof. Palumbo), "PLC pathways in cytoskeleton of human osteoblast/osteosarcoma cell and putative Ezrin correlation".
11:00-11:10 Dr. Tommaso Ferrari (tutor Prof. Recchia), "Cell line engineering to improve the recombinant adeno-associated virus (rAAV) production".
11:10-11:20 Dr. Francesca Pedrazzi (tutor Prof. Bianchi), "Mechanisms responsible for the deranged production of proinflammatory and profibrotic mediators in myelofibrosis".
11:20-11:30 Dr. Silvia Zacchino (tutor Prof. De Luca), "Development of a high-efficiency gene editing strategy by homology directed repair in human primary epidermal stem cells".

Session III: Oral Presentation XXXIX Cycle
Chairs: Prof. Elena Enzo, Prof. Elisa Bianchi, Prof. Silvia Belluti

11:30 - 11:40: Dr. Federico Corradi (tutor Prof. Recchia), "CRISPR-mediated base editing strategy for the correction of C-terminal dominant mutations in Rhodopsin gene".
11:40 – 11:50: Dr Elisa Bergamini (tutor Prof. Corradini), "Small Molecule, mRNA and Gene Therapy in Hemochromatosis and Ferroportin Disease".
11:50 - 12:00: Dr. Marica Malerba (tutor Prof. Manfredini), "Single-cell multiomic analysis of $CD34^{+}$ cells from myelofibrosis patients before and after JAK2-inhibitor treatment".
12:00 – 12:10: Dr. Giulia Raineri (tutor Prof. Clini, Prof. Corradini), "Single cell and spatial biology for the precise molecular profile of Idiopathic pulmonary fibrosis".
12:10 – 12:20: Dr. Giorgia Sinigaglia (tutor Prof. Quaglino), "Monocyte response to mitochondrial DAMPs in elderly subjects: correlation with chronic inflammation and atherosclerosis".

12:20 – 12:30: Dr Anita Neroni (tutor Prof. Manfredini), "Characterizing myelofibrosis stem cell for the identification of novel druggable targets".
12:30 – 12:40: Dr. Gaia Andrea Gozza (tutor Prof. De Luca, Dott. Enzo), "Towards a feeder-layer-free culture system for ex vivo combined cell and gene therapy of genodermatoses".
12:40 – 12:50: Dr. Ivana Jovanovic (tutor Prof. De Luca), "Molecular characterization of human epidermal stem and transient amplifying cells in homeostasis and wound healing".

13:00-13.30
PhD board meeting

Abstract Book

XXXVII Cycle

Student (name and surname)	**Elisa Adani**
PhD Cycle	**XXXVII**
Tutor	**Prof. Valeria Marigo**
Title of the project	**Contribution of metabolic dysregulations to photoreceptor cell death in Retinal Degeneration**

Abstract (do not exceed 250 words)

Retinitis pigmentosa (RP) is a group of inherited retinal dystrophies causing photoreceptors degeneration and blindness. RP shows extensive genetic heterogeneity, although common molecular events may significantly contribute to the disease progression.

RNA-Seq analysis performed on the cellular model 661W-A11 treated with Zaprinast to simulate rod degeneration highlighted significant downregulation of genes related to cholesterol biosynthesis and aerobic glycolysis. Additionally, the main activator of cholesterol synthesis SREBP2, displayed reduced nuclear localization after Zaprinast treatment, and cholesterol staining indicated altered intracellular distribution, suggesting disrupted metabolic homeostasis. In accordance with the *in vitro* model, cholesterol and glycolysis-related genes were downregulated also in the retinas of the two RP mouse models *rd10* and in $Rho^{P23H/+}$ at the peak of photoreceptor cell death. Real-time qPCR analysis of the $CD73^{+}$ enriched rod photoreceptor cell fraction from the $Rho^{P23H/+}$ murine model confirmed that the gene dysregulation was happening in photoreceptors.

To further validate metabolic dysfunction, we are now analyzing changes in cholesterol biosynthesis metabolites. In addition, we are evaluating gene changes and metabolic alterations in iPSCs-derived organoid models of photoreceptors degeneration to assess if the findings in murine retinas are relevant to the human pathology.

Based on the studies on murine models bearing different RP-causing mutations, we think that metabolic dysfunctions possibly occur during retinal degeneration regardless of the mutation and might be critical for rod survival. Further elucidations of the contribution of dysregulated metabolic processes to photoreceptor cell death could help in the identification of novel biomarkers and mutation-independent neuroprotective strategies.

Student (name and surname)	**Eleonora Cattin**
PhD Cycle	**XXXVII**
Tutor	**Prof. Alessandra Recchia**
Title of the project	**CRISPR/Cas9 system to correct laminopathic mutations in patients' cells: generation of *in vitro* models to identify biomarkers for Emery-Dreifuss Muscular Dystrophy and unravel pathogenic pathways in Hutchinson-Gilford Progeria**

Abstract (do not exceed 250 words)

To date at least 11 distinct disease phenotypes can be defined within the laminopathy group. Among this heterogeneous group, we are investigating type 1 Emery-Dreifuss Muscular Dystrophy (EDMD1) caused by mutations in *EMD* gene and Hutchinson-Gilford Progeria Syndrome (HGPS) caused by an aberrant splicing in *LMNA* gene and characterized by the presence of premature aging symptoms. EDMD1 is a rare genetic X-linked disease caused by the absence of the emerin protein. A cure for this disease is not available to date and the identification of biomarkers for the evaluation of disease progression is mandatory. To accomplish this aim, by using the CRISPR system, we genetically corrected myoblasts from 2 patients carrying a point mutation in the exon 1 of *EMD* gene, which abolishes the start codon or a 5-nt duplication in the exon 5 of the gene leading to a frameshift and generating a truncated protein missing the transmembrane domain. Hutchinson-Gilford Progeria Syndrome (HGPS) is one of the most severe disorders among laminopathies and share many symptoms with Mandibular hypoplasia, Deafness, Progeroid features and Lipodystrophy syndrome (MDPL) caused by *de novo* variants in *POLD1* gene. HGPS is caused by the c.1824C>T mutation in *LMNA* gene which leads to synthesis of progerin protein that accumulates at the nuclear envelope causing the disease phenotype. To correct this mutation in patient's myoblasts, we exploited the SpRY-HF1-ABE8e system. CRISPR-corrected cells from EDMD1, HGPS and MDPL patients will be instrumental to identify new therapeutic targets and unravel pathogenic pathways in HGPS.

Student (name and surname)	**Sara Gentile**
PhD Cycle	**XXXVII**
Tutor	**Prof. Francesca Fanelli**
Title of the project	**Insights into GPCR function from network analysis of all structures in the Protein Data Bank**

Abstract (do not exceed 250 words)

G protein coupled receptors (GPCRs) represent the largest protein superfamily in the human proteome and the targets of 30–40% of all drugs currently on the market. Structure network analysis is a cutting-edge tool to unravel GPCR function, which strongly relies on communication between the extracellular and intracellular poles of their structure.

We built the psnGPCRdb database (http://webpsn.hpc.unimo.it/psngpcr.php), which stores and compares the structure networks of all updated GPCR structures in the Protein Data Bank.

A pool of 478 Class-A structure networks from the psnGPCRdb was selected as a training set for Principal Component Analyses (PCA). The ability of PCA to assign receptor sub-family and G protein-signalling competence was verified on a test set of networks. The strength of metapath-links turned out to be the most effective network-based PCA-variable to distinguish inactive and active states, receptor sub-families, and G protein signalling.

The psnGPCRdb networks were also used to predict the hotspots in receptor-G protein interaction.

Independent of the functional state, all Class-A GPCRs exhibit communications between orthosteric and G protein binding sites. The G protein α-subunit homogenizes the metapaths on the receptors sides, taking on the task of determining signalling competence. In the G protein chimeras, at the C-term of the α-subunit, signalling competence depends on the structural communication in the whole Ras domain.

The structural communication-based models proved effective in assigning inactive-state GPCRs to a sub-family and active-state GPCRs to a signalling pathway as well as to predict the hotspot residues and their role in selective G protein signalling.

Student (name and surname)	**Matteo Marchionni**
PhD Cycle	**XXXVII**
Tutor	**Prof. Mattia Forcato**
Title of the project	**Overcoming Challenges in Cell Population Characterization, From Non-Spatial to Spatial Perspectives in Single-Cell Transcriptomic**

Abstract (do not exceed 250 words)

Single-cell omic technologies have revolutionized the ability to dissect complex tissues at a cellular level, enabling detailed classification of cell types and their molecular features. However, precise measurement of all transcripts at the single-cell level remains challenging due to high levels of technical and biological noise. As a result, identifying transcriptionally homogeneous yet biologically distinct subpopulations continues to be difficult, especially in certain populations and disease contexts.

Clear distinctions between cell populations are crucial for downstream analyses such as cell-cell interaction inference and gene regulatory network reconstruction, necessitating the development of novel bioinformatic solutions. To address these challenges, we introduce scPleiades, a novel R pipeline designed to improve cell classification for scRNA-seq and other single-cell omic data. scPleiades utilizes an innovative metric that enhances resilience to noise and improves the identification of distinct cell populations. Applied to publicly available scRNA-seq, scATAC-seq, and multiomic datasets from human cells, scPleiades demonstrates superior clustering accuracy and enhanced biological interpretability compared to existing methods.

Recent advances in spatial transcriptomics allow transcriptomic analysis at the single-cell or even subcellular level, providing unprecedented insights into tissue architecture. However, these technologies also introduce additional analytical complexities. While scPleiades addresses key challenges in traditional single-cell omics, the increased complexity of spatial transcriptomics demands more sophisticated approaches, including advanced quality control methods to detect artifacts and image-based analytical pipelines for accurate cell segmentation.

This work underscores the necessity for computational innovations to tackle the evolving challenges in single-cell and spatial transcriptomics.

Student	**Valentina Selleri**
PhD Cycle	**XXXVII**
Tutor	**Prof. Anna Vittoria Mattioli, Prof. Carlo Bortolotti**
Title of the project	**Telemedicine for health status monitoring and correlation with the inflammatory state, with a focus on mtDNA**

Abstract

The global population is aging due to advancements in public health, medical interventions, and technological innovations, including telemedicine. This technology enables the exchange of medical information across different locations using electronic communication devices, under a rigorous privacy control, and holds particular relevance for elderly or illness subjects by reducing travels and enabling continuous health monitoring.

The aim of my PhD research is to investigate the potential variations in vital parameters in different subjects populations, using ButterfLife device, and to explore the relationship between aging, chronic inflammation, and various pathological conditions, focusing on the role of circulating free mtDNA (cf-mtDNA). I applied telemedicine to healthy elderly subjects exhibiting symptoms associated with long-COVID, enrolled in an adapted physical activity program. Additionally, during the six-month research period in Valencia, I studied a cohort of obese patients, with and without metabolic syndrome (MetS), undergoing to six months of hypocaloric diet. I analyzed changes in vital parameters and the role of cf-mtDNA as a potential marker of inflammation. Data suggest that, especially in patients with MetS, mtDNA levels is correlated with vital signs and treatment outcomes. Beyond chronic inflammation, I also investigated the dynamics of acute inflammation, particularly in the context of traumatic brain injuries (TBIs), and the associated mtDNA-driven inflammatory and neuroinflammatory responses. Across all the conditions examined, mtDNA appears to be a key regulator of inflammatory cascade and, in combination with other circulating molecules, it may be a promising candidate biomarker for assessing individuals with chronic or acute inflammatory states.

XXXVIII Cycle

Student (name and surname)	**Matteo Bertesi**
PhD Cycle	**XXXVIII**
Tutor	**Prof. Rossella Manfredini**
Title of the project	**Chromosome 9p duplication enhances hematopoietic stem cells clonogenic potential and promotes immune escape in JAK2-mutant myeloproliferative neoplasms**

Abstract (do not exceed 250 words)

JAK2V617F is the most common mutation involved in the pathogenesis and diagnosis of Philadelphia-negative Myeloproliferative Neoplasms (MPN), a diverse group of clonal hematopoietic disorders causing an excessive production of mature blood cells through the hyperactivation of the JAK/STAT pathway.

This class of diseases is further exacerbated by additional co-occurring mutations and Copy Number Variations, that shape each patient's unique clonality.

Since JAK2 gene is located on chromosome 9 short arm (Chr9p), and JAK2V617F Variant Allele Frequency has a major impact on both severity and phenotype of the disease, any copy number abnormality involving its locus could be of clinical significance for disease evolution.

This work identified a distinct novel subgroup of MPN patients, characterized by chromosome 9 short arm trisomy (+9p) and allowing the study of the biological effects of chromosome 9 copy number abnormalities on JAK2-mutated MPN cells.

Single-cell studies allowed the elucidation of the molecular dynamics between chr9p amplification and JAK2 mutational burden, while a set of different molecular and functional assays gave insight into the selective advantage of circulating Stem and Progenitor Cells in +9p patients compared to JAK2V617F-homozygous and -heterozygous patients.

Remarkably, located downstream of JAK2 locus on Chr9 is CD274 gene, coding for the crucial immune checkpoint mediator PD-L1, which was found to be upregulated in +9p cells of the malignant clone. Immunofluorescence revealed also that PD-L1 protein relocalizes on plasma membrane in JAK2-homozygous and +9p MPN patients' monocytes, causing exhaustion of T cells in the immune system.

Student (name and surname)	**Matteo Corradini**
PhD Cycle	**XXVIII**
Tutor	**Prof. Carla Palumbo**
Title of the project	**PLC pathways in cytoskeleton of human osteoblasts/ osteosarcoma cells and putative Ezrin-correlation**

Abstract (do not exceed 250 words)

Osteosarcoma (OS) is one of the most common primary bone cancers in children and in young adults. The cancer molecular pathology is largely unknown, most diagnoses occur at advanced stage of disease, and metastases reduce patients' survival rate. Phosphoinositide pathway (PI) and related Phospholipase C (PLC) are involved in numerous physiological and neoplastic key-mechanisms of bone tissue development, such as proliferation-differentiation and cellular migration-invasion. The enzyme Phospholipase C (PLC) β1 plays a key role in osteoblast differentiation. PLCε can interact with small GTPases such as Rho and Ras which are involved in cell polarity, adhesion, motility, and cycle progression by regulating cytoskeleton rearrangements (in particular microfilaments and microtubules). Ezrin (EZR), a cellular membrane-cytoskeleton linker, often related to cellular invasion in several tumors, including OS, binds plasma-membrane thanks to the PI-P_2; possibly an interaction between EZR and PLCs may be established.

The aim of this project is to investigate whether the modulation of PLCs can impact OS cell growth, survival and migration by using U73122 inhibitor and his inactive analogue U73343.

Our results show how U73122 can significatively reduce the cell growth rate 24-48h after cell treatment compared to control and U73343. U73122 can downregulate all *PLCs'* gene expression 24h after single low dosage treatment. U73122 treatment reduces vimentin expression and increases p21 levels, while p53 is more variable towards the samples.

Student (name and surname)	**Tommaso Ferrari**
PhD Cycle	**XXXVIII**
Tutor	**Prof. Alessandra Recchia**
Title of the project	**Cell line engineering to improve the recombinant adeno-associated virus (rAAV) production**

Abstract (do not exceed 250 words)

Recombinant-AAV (rAAV) is a highly effective and safe vector for gene therapy, with several protocols approved by the FDA and EMA. However, the current vector production platform, triple transfection in HEK293 cells, might not meet the increasing demand. Thus, it is highly required to understand production bottlenecks and engineer the packaging cells to be more favorable and tolerant to vector production. We are exploiting the CRISPR Synergistic Activation Media (SAM) system to identify genes that enhance rAAV production in ReiCells, a clone of HEK293 cells routinely used by ReiThera s.r.l. to package vectors for clinical trials. To genome wide activate 23,430 genes, a rAAV GFP vector carrying a library consisting of 70,290 gRNAs was cloned and serially amplified in ReiCells expressing the SAM system (SAM-ReiCells) by lentiviral technology. SAM-ReiCells were characterized for vector copy number and for the expression of SAM components by ddPCR and flow cytometry. To test the activation system carried by SAM-ReiCells a reporter plasmid, with a gRNA targeting the β-Actin promoter, was generated.

NGS sequencing of the rAAV.gRNA.GFP genomes amplified in SAM-ReiCell identified gRNAs enriched during viral growth, ranking genetic targets whose upregulation, most likely, benefits AAV titer. To demonstrate an improved rAAV vector yield, the gRNA-related genes will be overexpressed one by one or in combination in ReiCells which will be challenged for the production of rAAV with clinical relevance.

Student (name and surname)	**Francesca Pedrazzi**
PhD Cycle	**XXXVIII**
Tutor	**Prof. Elisa Bianchi**
Title of the project	**Mechanisms responsible for the deranged production of proinflammatory and profibrotic mediators in myelofibrosis**

Abstract (do not exceed 250 words)

Myelofibrosis (MF) is a Philadelphia-negative neoplasm characterized by a nonresolving inflammation and bone marrow fibrosis fuelled by malignant hematopoietic cells that release abnormal quantities of proinflammatory and profibrotic mediators. We previously found that the expression of the proto-oncogene avian musculoaponeurotic fibrosarcoma (Maf) is increased in hematopoietic progenitors from MF patients. Given its role in the production of proteins involved in inflammation and fibrosis, we decided to better investigate Maf as a potential therapeutic target for MF.

For this purpose, drugs reported to reduce Maf protein levels were tested on monocytes purified from healthy donors (HDs). Among them Lanatoside C effectively decreased Maf protein levels. We are going to evaluate whether the expression levels of Maf target genes are similarly reduced upon treatment with Lanatoside C.

Additionally, transcriptome studies suggested an activation of the Interleukin-17A (IL17A) pathway in cells from MF patients. We therefore analyzed the expression of IL17A and its receptor (CD217) in mononuclear cells (MNCs) derived from patients with MF and HDs. We found that CD217 is expressed by monocytes, with no remarkable differences between MF patients and HDs. Interestingly, the fraction of IL17A-positive cells was remarkably higher in MF patients compared to HDs. IL17A-positive cells were indeed granulocyte precursors that are present in MF patients MNCs while almost absent in HD MNCs.

Due to the known proinflammatory role and potential profibrotic role of IL17A, the presence of a IL17A-producing cells in MF MNCs makes the IL17A pathway interesting for further investigation to assess its contribution in MF pathogenesis.

Student (name and surname)	**Silvia Zacchino**
PhD Cycle	**XXXVIII**
Tutor	**Prof. Michele De Luca**
Title of the project	**Development of a high-efficiency gene editing strategy by homology directed repair in human primary epidermal stem cells.**

Abstract (do not exceed 250 words)

Epidermolysis Bullosa (EB) is a rare severe genetic disorder characterized by skin fragility and blistering. Patients are exposed to recurrent risk of infections, dehydration, as well as constant pain. Although no definitive cure is available, emerging innovative treatments are being explored to expand the therapeutic options for EB using gene therapy. Among these, genome editing of epidermal stem cells holds a great promise.

To assess the feasibility of the CRISPR-Cas9 genome editing via Homology Directed Repair (HDR) using an Adeno Associated Virus (AAV) donor template, we performed preliminary studies in primary human keratinocytes.

We fused COL17A1, an EB-causing gene, to the GFP sequence, leading to the formation of a chimeric fluorescent protein detectable by flow cytometry and immunofluorescence analysis. Molecular analysis demonstrated correct donor integration at the genomic level, confirming the feasibility of the strategy. Employing an HDR-promoting drug, an editing efficiency of 85% was achieved.

To optimize the genome editing yield and safety related to Cas9 off-target activity and the HDR-promoting drug, studies are ongoing, with the goal of maintaining the epidermal stem cell compartment. Once identified the best editing conditions, further studies will focus on the design of different *ex vivo* gene editing strategies for EB-associated genes. The strategies will focus on inserting healthy coding sequences of the target genes within the corresponding genomic loci in primary epidermal stem cells. In this way, mutations that fall on a specific EB-gene could be addressed with a single molecular therapy, enabling the treatment of as many patients as possible.

XXXIX Cycle

Student (name and surname)	**Federico Corradi**
PhD Cycle	**XXXIX**
Tutor	**Prof. Alessandra Recchia**
Title of the project	**CRISPR-mediated base editing strategy for the correction of C-terminal dominant mutations in Rhodopsin gene**

Abstract (do not exceed 250 words)

Retinitis Pigmentosa (RP) is a group of hereditary disorders leading to vision loss due to the degeneration of rod and cones photoreceptor. Mutations in Rhodopsin (RHO) gene represent the most common cause of RP, responsible for the 25% of autosomal dominant forms. Within the most frequent pathogenic RHO variants in Europe are missense mutations involving the C-terminal proline in position 347, which affects the post-Golgi protein transport to outer segments (OS). We previously reported the development of a CRISPR/Cas9 strategy to knockdown the RHO P347S mutation, demonstrating the restoration of the correct localization to the OS in a mouse model of adRP treated with AAV-CRISPR.

My PhD project aims the development of a non-viral CRISPR adenine base editing (ABE) strategy to correct P347S and P347L mutations in Rhodopsin gene. This will provide a highly efficiently method to precisely correct the genetic defect without introducing DNA DSB.

HeLa cells were transduced with a lentiviral (LV) vector to stably express RHO-P347S and RHO-P347L mutations. SpRY-ABE8e, NG-ABE8e and Nme2-C-ABE8e base editors were tested *in vitro* along with different specific guide RNAs (gRNAs) transfecting HeLa clones with effector plasmids to assess the best combination to convert the target nucleotide. Editing analysis revealed that NG-ABE8e was highly efficient to correct C-terminal mutations without affecting RHO coding sequence.

Further experiments will be performed to evaluate efficiency and safety of a non-viral delivery of this platform in others cellular and animal models.

Student (name and surname)	**Elisa Bergamini**
PhD Cycle	**XXXIX**
Tutor	**Prof. Elena Corradini**
Title of the project	**Small Molecule, mRNA and Gene Therapy in Hemochromatosis and Ferroportin Disease**

Abstract (do not exceed 250 words)

Hereditary Hemochromatosis (HH) is a genetic disease characterized by systemic iron overload due to the loss of synthesis/activity of the iron hormone hepcidin and the juvenile form is known to be particularly severe. Ferroportin Disease (FD), which is caused by mutations in SLC40A1 gene, leads to iron retention in reticuloendothelial cells.

The aim of this PhD project is to target HH and FD with new therapeutic options including small molecules, mRNA and gene editing approaches that will be delivered by nanomedicine platforms.

Periferal blood mononuclear cells (PBMCs) from healthy donors were reprogrammed into induced pluripotent stem cells (iPSCs). iPSCs will be edited with several CRISPR/Cas9 strategies and differentiated into hepatocytes with the Forward Programming protocol in order to obtain in vitro models representative of the diseases of interest. Reprogramming into iPSCs and hepatic differentiation will be performed also on PBMCs from patients with Juvenile Hemochromatosis and Ferroportin Disease.

Hinokitiol (HK) was incapsulated in both cholesterol nanoparticles and nanostructured lipid carriers and calcein assays were performed on HepG2 cells to evaluate HK release from both formulations.

Potentially therapeutic mRNAs were designed in order to obtain maximum translational efficiency. mRNA constructs will be screened on HAMP KO HepG2 cells in order to select the most promising ones that will be delivered with specifically designed nanoparticles.

Several gene therapy approaches were designed using the CRISPR-Cas9 technology and will be loaded in nanoparticles.

Those therapeutic strategies will be evaluated for their efficacy on hepatocytes obtained from the differentiation protocol mentioned above.

Student (name and surname)	**Marica Malerba**
PhD Cycle	**XXXIX Cycle**
Tutor	**Prof. Rossella Manfredini**
Title of the project	**Single-cell multiomic analysis of CD34$^+$ cells from myelofibrosis patients before and after JAK2-inhibitor treatment**

Abstract (do not exceed 250 words)

Myelofibrosis (MF) is a myeloproliferative neoplasm (MPN) characterized by the acquisition of somatic mutations in hematopoietic stem cells, resulting in aberrant megakaryocytes proliferation, bone marrow (BM) fibrosis and extramedullary hematopoiesis. Most MPN patients harbor somatic mutations in genes involved in the JAK-STAT signaling and epigenetic regulation. The number and acquisition order of these mutations affect clonal evolution and response to targeted therapies, such as the JAK2-inhibitor Ruxolitinib, which provides symptomatic benefits, without improving BM fibrosis and survival.

The project aims to study the clonal architecture and immunophenotypic profile of a cohort of MF patients before and after Ruxolitinib treatment using single-cell multiomics. In light of this, we analyzed peripheral blood mononuclear cells and CD34$^+$ cells from both a Ruxolitinib-responsive and an unresponsive patient.

Clonal architecture reconstruction displayed that, after treatment, mutated clones in the responsive patient were reduced, whereas they expanded in the unresponsive one. Although both patients harbored epigenetic mutations, in the responsive patient the first mutation affected *JAK2* gene, while in the unresponsive patient the first mutational hit affected *ASXL1* gene. These results indicate that the mutational acquisition order may influence clonal evolution and response to therapy in MPNs. Single-cell immunophenotypic profiling showed that the Ruxolitinib-treated sample from the responsive patient was enriched in lymphocytes and natural killer cells, while the unresponsive patient's cells were enriched in granulocyte-monocyte progenitors and monocytes. Finally, the Ruxolitinib-unresponsive patient showed an expansion of the clusters harboring a higher number of mutations, while the lymphoid wild-type cluster became predominant in the responsive patient.

Student (name and surname)	**Giulia Raineri**
PhD Cycle	**XXXIX**
Tutor	**Prof. Enrico Clini, Prof. Elena Corradini**
Title of the project	**Single cell and spatial biology for the precise molecular profile of Idiopathic pulmonary fibrosis**

Abstract (do not exceed 250 words)

Idiopathic pulmonary fibrosis (IPF) is a rare, chronic, progressive, fibrosing interstitial lung disease (ILD) of unknown etiology, with a median survival of 3 years from the time of diagnosis. Besides the promising results of two antifibrotic drugs (Pirfenidone and Nintedanib) in slowing down the respiratory functional decline of IPF patients, an improvement in mortality rate has not yet been demonstrated. Since the histological pattern is not exclusively associated to IPF but with other conditions, a proven IPF diagnosis may represents a challenge for physicians. The aim of my PhD project is to face the clinical needs in pulmonary fibrosis pathogenesis, facilitating the diagnosis, thus improving the prognosis and shedding light on the molecular mechanism potentially crucial for the identification of new molecules for therapeutic approach.

The molecular characterization of transbronchial biopsies (TBS) and cryobiopsies (BLC) from IPF patients, and classified according to the extent of fibrosis, will be performed both through proteomic, RNA sequencing (bulk biopsy and scRNA-seq) and spatial transcriptomics. This approach will allow us to identify new biomarkers at the single cell level that could "individually" and specifically characterize the idiopathic form of pulmonary fibrosis for the development of new target drugs.

Student	**Giorgia Sinigaglia**
PhD Cycle	**XXXIX**
Tutor	**Prof. Daniela Quaglino**
Title of the project	**Monocyte response to mitochondrial DAMPs in elderly subjects: correlation with chronic inflammation and atherosclerosis**

Abstract

Aging is an important risk factor for atherosclerosis. Circulating free mitochondrial DNA (cf-mtDNA) acts as a Damage-Associated Molecular Pattern (DAMP), promotes the activation of innate immune cells, including monocytes, inducing proinflammatory cytokines production and plaque formation. Cf-mtDNA is sensed by immune cells via TLR9, inducing the expression of IL-6, TNFα, and IFNα, while cytosolic inflammasome component NLRP3 senses ox-mtDNA and promotes IL-1β and IL-18 maturation.

The aim of my PhD program is evaluating how cf-mtDNA activates monocytes by triggering an inflammatory response in elderly people, which pathways are activated and whether stimulation with mtDNA alone or in combination with proinflammatory stimuli modifies the metabolic features of monocytes.

This project involves the enrollment of 30 elderly subjects (>80 yrs) and 30 sex-matched young controls (<40 yrs). During my first year of PhD, I enrolled five elderly subjects and five young controls. I isolated monocytes from blood samples and treated cells with mtDNA alone and in combination with LPS. I first confirmed that monocytes from elderly people express TLR9 at intracellular level and that it colocalizes with cytoplasmic DNA. Then, I set up protocols for determining immunometabolic parameters using Seahorse technology in the same cells. Evaluation of inflammatory pathways activation on both resting and stimulated monocytes is ongoing.

Over the coming years, I will expand the cohort of subjects and I will continue to evaluate the inflammatory response of monocytes to inflammatory stimuli. Finally, I will correlate results with clinical parameters of atherosclerosis, as well as explore differences based on biological sex.

Student (name and surname)	**Anita Neroni**
PhD Cycle	**XXXIX**
Tutor	**Prof. Rossella Manfredini**
Title of the project	**Characterizing myelofibrosis stem cell for the identification of novel druggable targets**

Abstract (do not exceed 250 words)

Myelofibrosis (MF) is a myeloproliferative neoplasm characterized by splenomegaly, progressive anemia and ineffective hematopoiesis. A hallmark of MF is the perturbation of the JAK/STAT signaling pathway, which lead to clonal expansion of hematopoietic stem and progenitor cells (HSPCs).

A preliminary transcriptomic analysis was performed to identify new potential leukemic stem cell markers in $CD34^+$ $CD38^-$ hematopoietic stem cells (HSC) isolated from MF patients. Among the most over-expressed genes in MF HSCs, three transcripts were identified: *PROM1* (CD133), *CD9* (CD9), and *SLAMF4* (CD244). These markers were validated through flow cytometry, confirming that both $CD34^+$ and $CD34^+CD38^-$ cells from MF patients exhibit higher expression of CD133, CD9, and CD244 compared with HDs.

MF HSCs' functional characterization was initially focused on cells expressing CD9. $CD34^+$ HSPCs isolated from 9 MF patients and 7 HDs, were sorted based on CD9 expression using Fluorescence-Activated Cell Sorting (FACS). Afterward, purified $CD9^+$ and $CD9^-$ cells were functionally characterized through *in vitro* assays to evaluate their differentiation potential (multi-lineage assay in liquid culture) and clonogenic potential. $CD9^+CD34^+$ stem cells isolated from MF patients retained higher surface expression of CD34 over 12 days of liquid culture, compared to their $CD9^-$ counterparts. Moreover, a long-term culture (LTC) system was developed to quantify the frequency of HSCs capable of generating colonies after five weeks of culture (LTC-initiating cell, LTC-IC). These experiments revealed that MF $CD9^+$ HSCs include a greater number of LTC-ICs.

$CD9^+$ and $CD9^-$ HSC will next be transplanted in immunodeficient mice to study their ability to reproduce the disease.

Student (name and surname)	**Gaia Andrea Gozza**
PhD Cycle	**XXXIX**
Tutor	**Prof. Michele De Luca, Prof. Elena Enzo**
Title of the project	**Towards a feeder-layer-free culture system for ex vivo combined cell and gene therapy of genodermatoses.**

Abstract (do not exceed 250 words)

Autologous epithelial cultures are used in clinics to successfully produce epithelial grafts to treat burns or genetic skin diseases, such as Junctional Epidermolysis Bullosa (EB). We are now implementing new gene therapy approach to treat the recessive dystrophic type of EB (RDEB) and Lamellar Ichthyosis (LI).

The essential feature of a successful gene therapy is the presence of a correct amount of epithelial stem cells (EPSC) in the graft. This is now achieved using lethally irradiated mouse embryonic fibroblasts as a feeder layer, which preserve the long-term regenerative potential of EPSC during in vitro cultivation. To ease the complex culture procedure required to produce transgenic epithelial grafts, we aim at developing a fibroblasts-free stem cell-based gene therapy. This implementation will also lower production costs and reduce some quality controls required for the final product, making this advanced therapy more affordable.

To this end, we are studying the interplay between the murine fibroblasts and keratinocytes to identify the signaling required to sustain EPSCs self-renewal. Keratinocytes have been cultured onto clinical-grade fibroblasts and low-quality fibroblasts to assess their different influence on keratinocytes life span. The proteomic profile of the two types of fibroblasts has been compared. Preliminary results suggest that the amount of extracellular matrix produced by fibroblasts is critical to sustain the correct keratinocytes clonogenic potential. This knowledge will provide valuable insight for the development of an alternate fibroblast-free culture system able to reproduce the standard of quality reached by the classical culture method.

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PhD Cycle	**XXXIX**
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Title of the project	**Molecular characterization of human epidermal stem and transient amplifying cells in homeostasis and wound healing**

Abstract (do not exceed 250 words)

Epidermal stem cell-based regenerative medicine has proven to be lifesaving for patients suffering from massive full-thickness burns or affected by a genetic skin disease called Epidermolysis Bullosa (EB). To ensure long-term epidermal restoration, skin grafts require an adequate number of stem cells (KSCs), detected as long-lived, self-renewing holoclone-forming cells. However, KSCs represent only a small proportion of clonogenic keratinocytes and cannot be prospectively isolated. These cells give rise to a population of clonogenic short-lived, transient amplifying progenitors (TACs), eventually generating terminally differentiated keratinocytes. Due to the limited abundance of KSCs and challenges in their unequivocal identification and isolation there is an unmet need to define molecular markers of KSC compartment.

Recently, a distinctive molecular profile, called "holoclone signature", has been identified by microarray analysis and further confirmed through single cell gene expression profiles. Starting from these data, we will further characterize molecular pathways driving self-renewal in holoclone forming cells.

We constructed an *in silico* gene regulatory network based on the single cell gene expression of clonogenic keratinocyte cultures and critical candidate genes were detected as potential hallmarks of the holoclone signature, while not in other clonal types. Using *in vitro* models of said genes KO in human primary keratinocytes, we intend to further investigate the biological role of these potential key regulators in holoclone forming cells.