# Abstract list National Forum On Precision Medicine

# SESSION 1.1

# Exploring the role of NRF1 in Multiple Myeloma through characterization of its transcription factor regulation profile

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Multiple Myeloma (MM) is the second most common hematological disease characterized by uncontrolled proliferation of plasma cells and excessive production of immunoglobulins. Despite the advances in treatments and the efforts to comprehend MM progression mechanisms, it remains a complex and challenging disease. Understanding the interplay of transcription factors activity and epigenetic changes is a crucial step in unveiling the multilayered pathogenesis of MM.

Using ATAC-seq, we dissected the chromatin accessibility landscape of a cohort of 55 MM patients, including those collected at diagnosis and after therapy. Then, we built a computational workflow and performed footprinting analysis to decipher the TFs occurrence within the accessible loci. We defined a subset of TFs bound at the most penetrant loci of our cohort and identified NRF1 as the most enriched. We investigated the binding of NRF1 in MM cell lines and determined a consensus binding set of 5749 sites that were strongly bound by NRF1 at diagnosis, but not in pre-malignant state.

We identified a subset of 103 genes whose activity is linked with aggressive disease and adverse outcomes, l leveraging data from 457 patients from the CoMMPASS dataset. This signature allowed the identification of 195 patients with poorer prognosis (20 months) compared to 262 others. The signature is strongly enriched in ubiquitination, a process that promotes MM cell survival. ConsistentlyConsistently, NRF1 depletion results in decreased protein ubiquitination alongside a concurrent increase in ER stress.

Subsequentially, we observed an increase of NRF1 in MM cell lines after time course at different doses of Bortezomib (BTZ). Then, we established primary MM cell lines models which recapitulates BTZ resistance and performed scRNAseq, detecting high NRF1 expression in one sub population of resistant cells.

# From dysbiosis to gut permeability dysfunction: new biomarkers and new therapeutic strategies for stratification and reduction of cardiovascular risk

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The intestine represents the largest and one of the most crucial internal barriers in the body, shielding the host from harmful substances and microorganisms within the gut lumen. The symbiotic microbiota in the intestines plays a crucial role in forming the protective barrier of the intestinal mucosa. In the last decade, it has emerged that gut permeability may be a risk factor for cardiovascular and cancer diseases, contributing to inflammatory and oxidative stress sequelae and subsequent disease progression.

To early identify pathological disorders and improve therapeutic strategies regarding dysbiosis-related gut permeability, Spoke 5 and Spoke 1 are collaborating for the following objectives.

Objective 1: a) evaluation of the impact of the immune system-microbiome interaction on gut permeability in vitro. Human ex vivo isolated immune cells (from peripheral blood, lamina propria and tumor infiltrating lymphocytes) are cocultered with commensal or pathogenic microbial ecologies, or with their metabolic products, and the functional effects on epithelial cells permeability are evaluated in vitro; b) evaluation of the impact of the immune system-microbiome interaction on gut permeability in murine models. Patient's murine avatars will be reconsitutted with patients' derived microbiomes and in vivo effects of immune-system-microbiome interaction will be evaluated in vivo.

Objective 2: a) evaluation of circulating biomarkers of altered gut permeability. Analysis in 3.455 subjects allocated to "Moli-sani-Study" (that is a cohort study aiming at evaluating the risk factors such as environmental, genetics, or biomolecular, linked to chronic-degenerative disease with particular regard to cancer and cardiovascular disease) of two biomarkers of gut permeability such as zonulin and LPS and oxidative stress status by NOX2 activity; b) investigation of molecules able to reduce gut permeability. In vitro analysis of the protective effect of natural molecules (for example polyphenol extracts) or small molecules (for example inhibitors of NOX2 activation) on Caco2 cells subjected to a damage.

Objective 3: Developing nutritional interventions aimed to limit proliferation of LPS-producing E. coli and enterobacteriaceae. The capability of E. coli and enterobacteriaceae to growth on different prebiotics has been investigated, in order to formulate a prebiotic mixture potentially able to limit proliferation of these pathobionts. The prebiotic mixture has been tested in the atherosclerosis mouse model Apoe-/-, with or without the supplement of red rye, to determine whether the changes of microbiota composition associated with prebiotic and/or red rye treatment can affect: a) microbiota composition; b) gut permeability; c) development of atherosclerosis.

Objective 4: Identification through proteomics analysis of novel biomarkers associated with microbiome alterations in a mouse model of atherosclerosis. a) Validation of the mouse Apoe-/- atherosclerotic model by assessing cholesterol levels and ultrasonographic analysis to monitor the formation of atherosclerotic plaques. b) Proteome profiling of target tissues of wt or Apoe-/- animals supplemented with prebiotic substances or red rice extract alone and in association with the prebiotic mix.

SPOKE 5, TASK 3.1 and SPOKE 1, TASK 3.1

# From extended trailers to clinical proofs of concept. The case of alkaptonuria

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Alkaptonuria (AKU) is a rare autosomal recessive metabolic disorder caused by mutations of homogentisate 1,2-dioxygenase (HGD) gene which affect phenylalanine and tyrosine catabolism. This leads to a deficiency of the HGD enzyme with consequent accumulation of homogentisic acid (HGA) in body fluids and different tissues, inducing a multisystemic and highly debilitating disease whose main features are dark urine, ochronosis (HGA-derived melanino-like pigments), and a severe form of osteoarthropathy. It's generally associated to other clinical manifestations including heart valves, kidneys, and prostate damage and comorbidities, such as secondary amyloidosis (SA).
The main symptoms of the disease appear around the third decade of life, but a proper diagnosis is often delayed due to the lack of a standardized methodology and knowledge among physicians. Thus, the clinical management of AKU needs to be improved and the main mechanisms involved in its pathogenesis should be better investigated for the development of new diagnostic tools for early detection and patient-tailored therapies.
Since the low incidence of this pathology, it is very important to implement with proteomics and metabolomics data an already established digital platform for patients with alkaptonuria, that already includes genetic, biochemical, histopathological, clinical and quality of life information. The study will lead to the identification of potential AKU-specific biomarkers associated with different genetic characteristics of patients such as the SAA1 protein polymorphism and help shed light on some of the biological mechanisms underlying the secondary amyloidosis implicated in this disease.
The second aim focuses on the setup of an innovative flow dynamic in vitro model of AKU to investigate the molecular mechanisms associated to the HGD misfunction. To this purpose, HGD gene knockout (HGD-KO) has been developed in hepatocyte (HepG2) and chondrocyte (C20A4) cell lines using CRISPR/Cas9 technology. Clones lacking HGD enzyme have been selected by limiting dilution and Western blot analysis. The effective silencing has been confirmed by Sanger gene sequencing. The obtained results revealed the presence of different frameshift mutations in three selected HepG2 clones in comparison to the control line, confirming that the HGD-KO has been reached. Concerning chondrocytes, the sequencing analysis on three selected clones is ongoing. Cell viability, proliferation, apoptosis and production of reactive oxygen species have been assessed in these HGD-KO clones.

# The effect of luteinizing hormone (LH) in modulating Anti-Müllerian hormone (AMH) pathway in human granulosa cells

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Anti-Müllerian hormone (AMH) is a member of the TGF-beta superfamily. It binds its specific receptor, AMHR2, and its transduction mechanism involves the canonical SMAD pathway. In particular, phosphorylated SMAD 1, 5 and 8 interact with SMAD 4 forming a complex that promotes the expression of AMH target genes. AMH is produced by fetal testis Sertoli cells and, as the name implies, leads to the regression of the Müllerian ducts in male fetus. It is also expressed in female gonads since the 36th week after conception and its levels fluctuate during ovarian cycle, reaching the peak in pre-antral and small antral follicles. Nowadays is common knowledge that AMH acts as an inhibitor of the primordial follicle recruitment, preserving the primordial follicle pool from premature depletion. AMH levels in serum, indeed, are commonly used in clinical practice to estimate women ovarian reserve. However molecular mechanisms underlying AMH regulation of follicles activation are still unclear. Little is known also about the interplay between human gonadotrophins (FSH and LH) and AMH. While, in vitro studies showed that FSH increases AMH expression, information about the relationship between LH and AMH are lacking. Recently, it has been demonstrated that functional LH receptors are expressed also in smaller follicles, albeit in small quantity, during the phase of follicular growth that traditionally has been considered gonadotrophin independent. This evidence led us to hypothesize that LH may play a role in both early folliculogenesis and follicle recruitment by interacting with AMH pathway in granulosa cells. We performed our in vitro experiment on primary human granulosa cells (hGCs). We demonstrated that LH treatment reduced AMHR2 mRNA expression through RT-PCR. The negative modulation was also observed through western blotting assay in AMHR2 protein expression. In order to study the effect of LH on AMH pathway, we analyzed the phosphorylation of SMAD 1 and 5 after AMH treatment. Interestingly, SMAD phosphorylation has been reduced in LH pre-treated hGCs. We performed the experiments also in hGCs silenced for LH receptor expression. As expected, without LH receptor, LH treatment was ineffective in inhibiting AMHR2 expression. AMH expression, meanwhile, was not affected by LH treatments. Our results show for the first time that LH exposure impacts the AMH pathway in hGCs. This evidence led us to suggest that LH may have a potential role in primordial follicle activation by inhibiting the preserving role of AMH. Further studies may contribute to clarify the translational potential of LH-AMH interplay as a future target in infertility treatment.

# DNA topoisomerase I poisons trigger R-loop-mediated micronuclei enhanced by transcription factor IIS mutations

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Cancer cells are characterized by a high transcription levels, often called “hyper-transcription”. DNA topoisomerase I is a critical factor during transcription elongation. However, topoisomerase I can also contribute to cancer genome instability. During catalytic activity, topoisomerase I forms a transient intermediate, topoisomerase I-DNA cleavage complex (Top1cc), to allow strand rotation and duplex relaxation, which can lead to high levels of DNA-RNA hybrids and micronuclei. Top1cc levels are increased by anticancer Topoisomerase I poisons, such as camptothecins, used in standard chemotherapies of human lung, ovary and colon cancers. To comprehend the relevant aspects of molecular mechanisms, we have integrated genomic data of Top1cc-triggered hybrids and double-stranded DNA breaks (DSB) shortly after Top1cc induction by DRIP-seq and END-seq technologies. The results have revealed that Top1ccs increase hybrid levels with different mechanisms. DSBs are at highly-transcribed genes in early-replicating initiation zones, and overlap with hybrids downstream of accumulated RNA polymerase II (RNAPII) at gene 5’-ends. A transcription factor IIS mutant impairing transcription elongation further increased RNAPII accumulation likely due to backtracking. Moreover, Top1ccs can trigger micronuclei when occurring during late G1 or early/mid S, but not during late S. As micronuclei and transcription-replication conflicts are enhanced by transcription factor IIS mutations, our results support a role of RNAPII arrest in Top1cc-induced transcription-replication conflicts leading to DSBs and micronuclei. The data show that specific mutations and genes of transcription elongation factors can influence the genome instability and activity of anticancer DNA topoisomerase poisons.

# First-hit SETBP1 mutations cause a myeloproliferative disorder with bone marrow fibrosis mimicking triple negative myelofibrosis

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Somatic SETBP1 mutations are recurrent in both myeloproliferative neoplasms (MPN) and myelodysplastic syndromes (MDS). To characterize the early steps of SETBP1-mediated leukemogenesis, we generated a conditional mouse model expressing SETBP1G870S mutant in the entire hematopoietic tissue. Heterozygous SETBP1G870S mice showed evidence of accumulation of white blood cells with a marked imbalance between the lymphoid and myeloid lineages in favor of the latter, characterized by an increase in mature myeloid cells in absence of circulating blasts or non-segmented myeloid precursors. Kaplan-Meier analysis revealed a dramatic decrease in event-free survival in SETBP1G870S mice. Mice were characterized by hepatosplenomegaly with massive infiltration by myeloid elements, disruption of normal tissue architecture and signs of extramedullary hematopoiesis. Bone marrow (BM) histology showed overt myeloid hyperplasia with fibrosis and no evidence of dysplasia except for the megakaryocytic lineage.
Single-cell RNA-sequencing (scRNA) on BM Lin- cells identified Spi1 as one of the most upregulated genes; it is known that Spi1 regulates PU.1 which in turn promotes the maturation of bone marrow early precursors towards the granulocytic/monocytic lineages by directly impairing the transcription of Gata2 and Gata1. In line with these data, Gata2 and Gata1 expression was profoundly suppressed in the early myeloid precursors of SETBP1G870S mice, which associated with the down-modulation of markers of the erythroid lineage, such as the Carbonic Anhydrase 1, in the MEP differentiation branch.
Since our mouse model recapitulates many clinical features of primary myelofibrosis (PMF), we set out to assess SETBP1 mutations in the context of PMF that are triple-negative for the classical JAK2, CALR and MPL mutations (TN-PMF). We analyzed 36 TN-PMF patients by exome sequencing and in 7 cases (19.4%) high VAF SETBP1 degron mutations were identified. A markedly reduced overall survival was observed for SETBP1 positive patients, with a median survival time of 24 months (median survival not reached at 60 months for SETBP1 negative patients). To dissect the clonal architecture of SETBP1 positive TN-PMF at single-cell resolution, we applied single-cell targeted DNA sequencing on 3 SETBP1-mutated TN-PMF samples: in all cases we identified SETBP1 as a very early clonal event.
In conclusion, we show that the SETBP1G870S mouse model recapitulates all the aggressive clinical features also induced by SETBP1 in primary myelofibrosis, such as leukocytosis without differentiation block or myeloid dysplasia, BM fibrosis with dysplastic megakaryocytes and progressive splenomegaly.

SPOKE 3, TASK 4

# Interaction of radiopharmaceuticals with somatostatin receptor 2 revealed by molecular dynamics simulations

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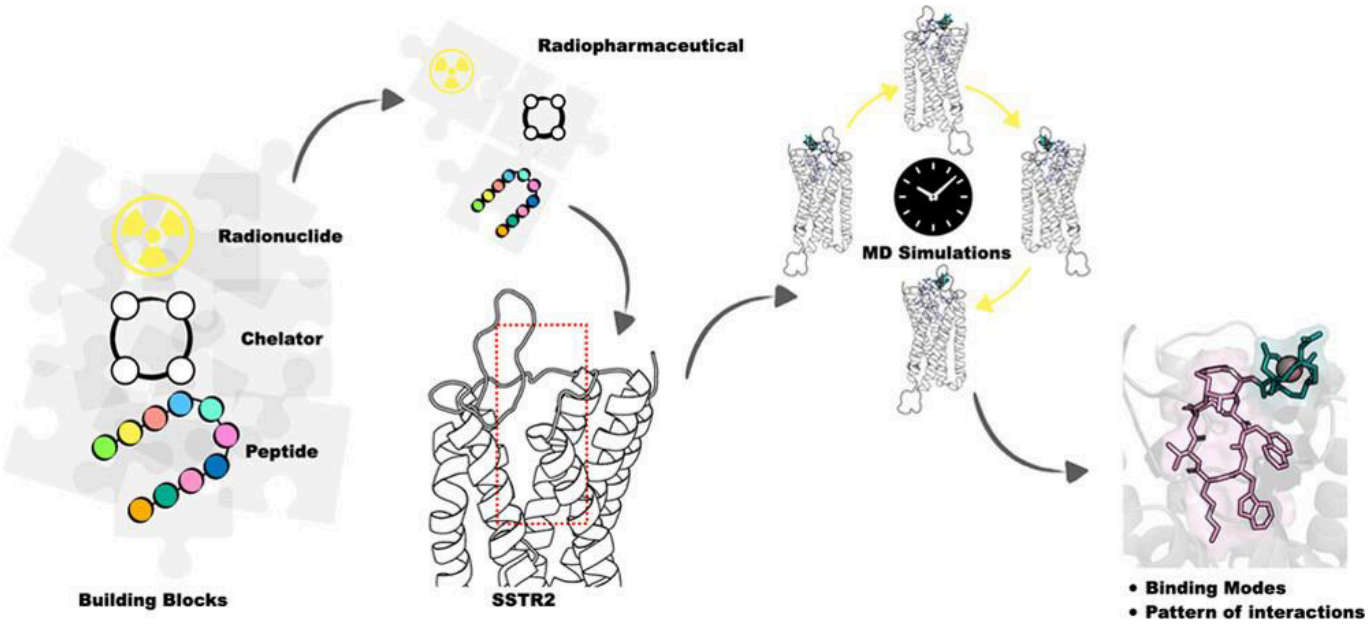
Cancer cells are characterized by a high transcription levels, often called “hyper-transcription”. DNA topoisomerase I is a critical factor during transcription elongation. However, topoisomerase I can also contribute to cancer genome instability. During catalytic activity, topoisomerase I forms a transient intermediate, topoisomerase I-DNA cleavage complex (Top1cc), to allow strand rotation and duplex relaxation, which can lead to high levels of DNA-RNA hybrids and micronuclei. Top1cc levels are increased by anticancer Topoisomerase I poisons, such as camptothecins, used in standard chemotherapies of human lung, ovary and colon cancers. To comprehend the relevant aspects of molecular mechanisms, we have integrated genomic data of Top1cc-triggered hybrids and double-stranded DNA breaks (DSB) shortly after Top1cc induction by DRIP-seq and END-seq technologies. The results have revealed that Top1ccs increase hybrid levels with different mechanisms. DSBs are at highly-transcribed genes in early-replicating initiation zones, and overlap with hybrids downstream of accumulated RNA polymerase II (RNAPII) at gene 5’-ends. A transcription factor IIS mutant impairing transcription elongation further increased RNAPII accumulation likely due to backtracking. Moreover, Top1ccs can trigger micronuclei when occurring during late G1 or early/mid S, but not during late S. As micronuclei and transcription-replication conflicts are enhanced by transcription factor IIS mutations, our results support a role of RNAPII arrest in Top1cc-induced transcription-replication conflicts leading to DSBs and micronuclei. The data show that specific mutations and genes of transcription elongation factors can influence the genome instability and activity of anticancer DNA topoisomerase poisons.



# TXNIP is a novel substrate of the E3 ubiquitin ligase WWP1 in cellular redox state regulation

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WWP1 is a HECT-type E3 ubiquitin ligase, overexpressed in acute myeloid leukemia (AML). Its oncogenic activities consist in conferring a proliferative advantage to leukemic blasts and counteracting apoptotic cell death and differentiation. Here we identified thioredoxin-interacting protein (TXNIP) as a novel WWP1 substrate, which negatively regulates thioredoxin (Trx) bioavailability and prevents its disulfide reductase function. Pull-down assays revealed that WWP1 directly interacts with TXNIP and ubiquitination assays showed that wild-type WWP1 (WT), rather than an enzymatically inactive WWP1 mutant (C890A), is capable to induce TXNIP poly-ubiquitination thus promoting its ubiquitin-dependent proteasomal degradation. Indeed, WWP1-mediated TXNIP ubiquitination was prevented in the presence of the K48R ubiquitin mutant, while it was unaffected in K63R ubiquitin mutant expressing cells, implicating K48 in polyubiquitin chain formation by WWP1. Together with the accumulation of TXNIP observed in WWP1-depleted cells, this result confirms that WWP1-dependent ubiquitination of TXNIP leads to its proteasomal degradation.
Moreover, we observed that in response to WWP1 inactivation, the accumulation of TXNIP reduces Trx activity and increases reactive oxygen species (ROS) production. As a consequence, WWP1-depleted cells present increased levels of DNA strand breaks and subsequent apoptosis. Notably, WWP1-dependent accumulation of DNA damage is reversed by treatment with the antioxidant N-acetyl cysteine (NAC), a ROS scavenger.
Coherently with TXNIP stabilization following WWP1 inactivation, we also observed an impairment of glucose up-take and consumption, further confirmed by the reduced gene expression of the glucose transporters GLUT1 and GLUT4, and of the enzymes involved in glucose metabolism, such as lactate dehydrogenase A and B, which are known to be regulated by TXNIP. WWP1 downregulation significantly reduced glycolysis and oxidative phosphorylation measured by seahorse. Overall, these findings indicate decreased functional glycolysis in the absence of WWP1. Hence, a contribution to increased cell death observed in WWP1-depleted cells, is also possibly arising from the attenuation of glucose up-take and glycolytic flux resulting from TXNIP accumulation.
In conclusion, our results identify TXNIP as a novel substrate of WWP1 and suggest that TXNIP reduced availability, as a result of WWP1 overexpression, would promote proliferation and survival of AML blasts, thus fostering cancer progression.

SPOKE 1, TASK 3.2

# The WWP1-JARID1B axis sustains chemoresistance of acute myeloid leukemia

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Resistance to genotoxic drugs due to increased DNA damage repair is the leading cause of chemotherapy failure in acute myeloid leukemia (AML) patients. The E3 ubiquitin ligase WWP1 is an oncogenic protein, overexpressed in several cancers including AML, in which it exerts its protumorigenic activity by promoting cancer cell proliferation and survival. Here, we found that WWP1 inactivation sensitizes AML cells to the cytotoxic activity of DNA damaging chemotherapeutic drugs. To assess whether WWP1 depletion increases the sensitivity of AML cells to genotoxic compounds by affecting DNA repair, we measured DNA strand break repair kinetics following WWP1 silencing. We observed a significant delay in the clearance of DNA strand breaks in WWP1-depleted relatively to control cells. As a result of WWP1 inactivation, we also observed an impaired efficiency of both nonhomologous end-joining (NHEJ) and homologous recombination (HR) systems. The reduced DNA damage repair capability of WWP1-depleted cells resulted from a defective recruitment of repair proteins to the site of DNA damage. In an attempt to identify novel substrates of WWP1 implicated in DNA repair, we have conducted a proteomic analysis (UbiScan) that pinpointed the istone lysine demethylases KDM5B/JARID1B as a candidate target of WWP1. Of note, the demethylase activity of JARID1B is necessary for efficient recruitment of several DNA damage repair factors and resolution of DNA damage. Coherently, genetic or pharmacological inhibition of JARID1B increase the sensitivity of cancer cells to chemo- and radiation therapies. We next validated JARID1B as an interactor and a substrate for the ubiquitination activity of WWP1 and found that WWP1 positively regulates JARID1B half-life and protein stability. As a result, downregulation of JARID1B rising from WWP1 inactivation was associated with enhanced global levels of H3K4 tri-methylation (H3K4me3) and with increased H3K4me3 enrichment at both promoter and within gene body regions of JARID1B target genes, as assessed by ChIP-sequencing. Furthermore, we observed transcriptional activation of JARID1B target genes in WWP1-depleted relatively to control cells. All together, these data identify JARID1B as a bona fide target of WWP1 and imply that WWP1-mediated regulation of JARID1B impacts on its ability to modify chromatin and to recruit DNA damage repair factors, thus ultimately affecting chemosensitivity of AML cells.

SPOKE 1, TASK 3.2

# Investigating molecular pathways in colorectal cancer: developing advanced cellular models to study tumor aggressiveness

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Morphological features, such as tumor buddings (TBs), poorly differentiated clusters (PDCs) and micropapillary structures (MIPs), are established negative prognostic markers of aggressive colorectal cancer (CRC), exhibiting cancer cell dissociation and dissemination. Cancer cells within these tumor types undergo partial or full epithelial to mesenchymal transition (EMT), enabling them to gain enhanced migratory and invasive capabilities, which contributes to increased tumor aggressiveness and metastatic potential. Existing data define CRC tumors with a mesenchymal phenotype as corresponding to the CMS4 molecular subgroup, which is characterized by the upregulation of EMT pathways, TGF-β signaling, matrix remodeling, stromal infiltration, and poor relapse-free survival.
We recently demonstrated that the splicing signature of the NF-YA gene, which encodes for the regulatory subunit of the transcription factor NF-Y, can stratify patients on the basis of CMS subtypes. Higher expression of the NF–YAI transcript variant distinguishes the CMS4 group and correlates with the expression of EMT, extracellular matrix, and cell adhesion genes.
Based on these results, the role of NF-YAI in conferring aggressiveness to MIPs was investigated. Using RNA-FISH hybridization on tissue sections derived from patients with MIPs, we found that NF-YAI shows a higher expression and a characteristic distribution, compared to colon adenocarcinomas. Cellular models overexpressing NF-YA isoforms were generated and studied in vitro. In particular, we investigated the interaction between NF-YAI-expressing cancer cells and host cells of the tumor microenvironment, specifically focusing on two key components: tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs). 2D and 3D co-culture experiments identified a key role for NF-YAI in fostering cell interactions within the tumor niche and establishing a positive metastatic feedback mechanism.
We are currently performing spatial transcriptomic analysis on samples from patients with MIPs to identify additional biomarkers for the development of new cellular models for aggressive CRC.

# Gene expression pattern predictive of ovarian follicle development and maturation

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In women undergoing assisted reproduction techniques (ART), poor response to ovarian stimulation leads to low oocyte yield and is related to the patient's genetic background. We compared the expression levels of genes regulating follicular development, as well as the intrafollicular testosterone and estradiol levels, between small (SFs, diameter<10 mm) and large ovarian follicles (LFs, diameter>16 mm) from ART women. We aim to identify markers of follicle maturation. Granulosa cells (hGLC) and follicular fluids from SFs and LFs were collected from twenty-two patients undergoing ART. Gene expression analysis on hGLC was performed by digital droplet PCR, while testosterone and estradiollevels were measured by homogeneous time-resolved fluorescence. Results were matched with clinical parameters by principal component analysis (PCA) and statistical analysis was performed using Mann-Whitney's U-test ($p<0.05$). We found higher expression levels of FSHR, GPER, AMHR2 and CCND2 genes in SFs compared to LFs, while no differences were found for CYP19A1, LHCGR, XIAP and TP53 gene expression. Moreover, FSHR/GPER expression ratio was lower in LFs than SFs, and positively correlated to CCND2 expression levels only in SFs, reflecting that they are involved in follicle development. PCA analysis revealed that GPER and FSHR expression levels discriminate different populations of SFs. Higher testosterone levels were detected in LFs versus SFs, while no different estradiol levels were found, reflecting higher estrogenic potential of small than large antral follicles. PCA analysis demonstrated that ART protocol and drug used for clinical treatment did not impact results. In summary, SFs and LFs have different gene expression pattern according to the maturation state.

# Coping with antibiotic resistance via ligand- and structure-based strategies

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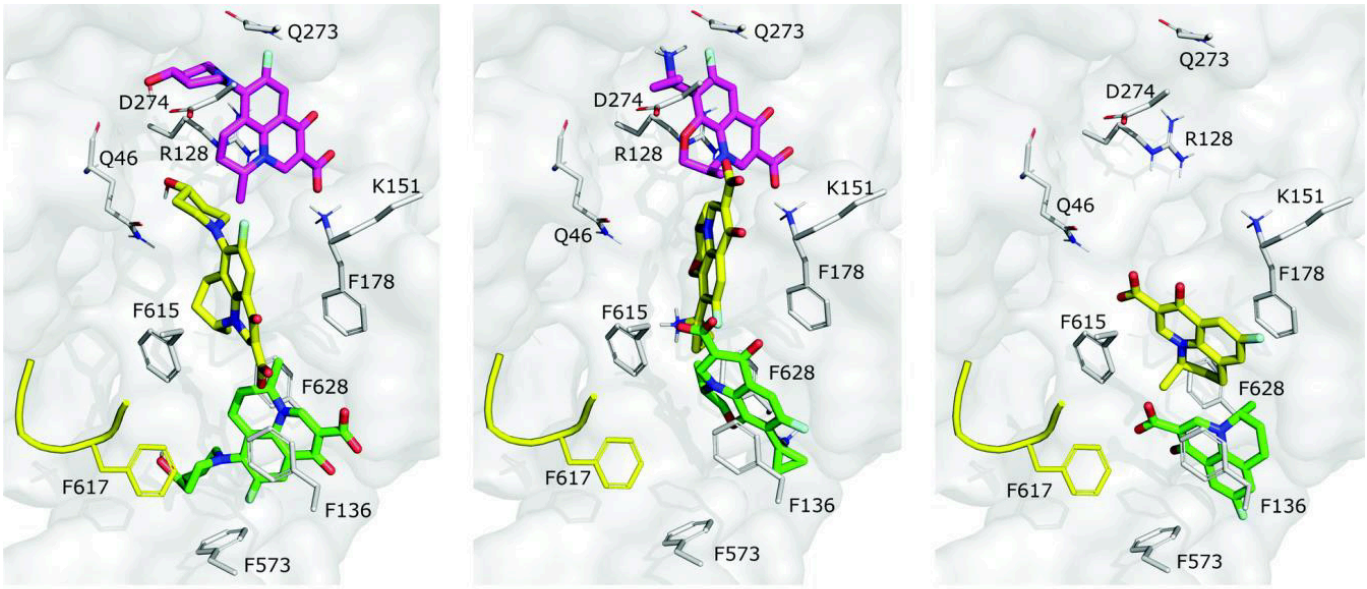
The increasing spread of antibiotic resistance in clinics is causing a global health crisis. Gram-negative bacteria, such as Pseudomonas aeruginosa, are particularly challenging due to the presence of an outer membrane reducing the permeability of antimicrobials and of efflux pumps that expel drugs outside the cell. Given the complexity of these biological systems, holistic approaches able to consider multiple factors contributing to antimicrobial resistance are needed.

We present two ongoing computational projects contributing to the challenge of antimicrobial resistance. The first one, AB-DB, is an open database of all-atom force-field parameters, molecular dynamics trajectories, quantum-mechanical properties, and curated physico-chemical descriptors of antimicrobial compounds. The collection stores more than 300 molecules belonging to 25 families including the most relevant antibiotic classes in clinical use.

In the second project, we performed a systematic ensemble docking campaign coupled with cluster analysis and molecular-mechanics optimization of docking poses to investigate the interaction between quinolone antibiotics and MexB, the drug/proton antiporter of the major efflux pump MexAB-OprM in P. aeruginosa. Our study reveals different binding preferences of (fluoro)quinolones towards the sub-sites of the large deep binding pocket, supporting the hypothesis that MexB substrates oscillate between different binding modes




Predicted binding modes of (A) norfloxacin, (B) pazufloxacin and (C) flumequine. Binding Modes 1, 2, and 3 are green, yellow, and magenta, respectively. Binding Mode 3 was not found in case (C). The switch loop is represented as a yellow cartoon.

# Medullary colon carcinoma (MCC) molecular characterization

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Medullary colon carcinoma is a rare subtype of colon cancer usually associated with loss of ARID1A, microsatellite instability and strong correlation with BRAF V600E mutation.
We retrospectively collected eight medullary carcinomas resected from 2020 to 2023 at the Mediterranean Institute of Oncology. Six of these were treated inhouse while two patients were lost at follow up.
We evaluated immunohistochemical expression of ARID1a, MLH1 and PMS2, calretinin and CDX2 in these samples.
In addition, the 8 samples were evaluated using Amoy's HANDLE Classic NGS diagnostic panel (ILLUMINA). This panel allows analysis of 36 driver genes associated with colon and lung cancers, evaluating SNVs, InDels, fusions, and CNVs from FFPE tissue.
All patients but one were female, with median age of 75,3 years and 7 cases were located in the right colon.
All medullary carcinomas were morphologically characterized by a solid growth pattern with poorly differentiated non-glandular, solid sheets of eosinophil neoplastic cells with a significant amount of tumor-infiltrating lymphocytes.
Immunohistochemistry (IHC) was performed on all tumor sections showing loss of expression of ARID1a, MLH1, PMS2 and CDX2 with at least focal expression of Calretinin.
NGS sequencing and IHC showed that among the 8 patients:
• 6 (75%) exhibit a pathogenic mutation in BRAF exon 15 (c.1799T>A:p.(V600E)).
• 4 (50%) have a variant with uncertain significance in FGFR4 exon 9 (c.1162G>A:p.(G388R)).
• 2 (25%) show a pathogenic mutation in TP53 exon 5 (c.455C>T:p.(P152L)
• 8 (100%) have MSI and loss of expression of ARID1A
This study confirmed that medullary carcinoma is frequently located in the right colon and occurs more in older female patients. Medullary carcinomas are associated with microsatellite instability, especially with loss of expression of MLH1 and in this study, we also confirmed the strong correlation with BRAF mutation (V600E).
Despite the morphological and immunohistochemical homogeneity, the NGS evaluation of these tumors showed heterogeneous results. Further studies are needed to elucidate the biological characteristics of medullary carcinomas and ARID1A role as a diagnostic and prognostic marker in order to individualize the therapy for patients affected by this disease.

# Exploring potential VEGF receptor 2 inhibitors: a molecular modeling and pharmacophore-based screening approach

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The vascular endothelial growth factor (VEGF) receptor 2, a membrane tyrosine kinase receptor, together with its native ligand VEGF-A, triggers endothelial cell proliferation, migration, survival, and the formation of new blood vessels - all crucial functions for angiogenesis. With the aim of identifying additional new ligands or acquiring novel information to craft potent drugs, the focus of this study was to point to this macromolecule as to a possible target for selective modulators or inhibitors. Using the Pharmit server, we achieved pharmacophore-based screening on libraries containing about 450 million compounds, resulting in a set of ligands/inhibitors, which were tested using molecular modeling tools such as molecular docking, molecular dynamics, and three-dimensional quantitative structure–activity relationship analysis. The best stable complex obtained, the PubChem-143070699/hiVEGFR2 complex, perfectly matches the proposed pharmacophore model (an integration of previous models), demonstrating a nanomolar affinity. While experimental evidence remains mandatory to decipher the mechanisms underlying VEGFR2 inhibitors, the structural insights gleaned from this research hold promise for advancing the development of increasingly potent and precisely targeted therapies for VEGFR-associated conditions, such as cancer and fibrosis-related diseases.

# Investigating molecular pathways in colorectal cancer: developing advanced cellular models to study tumor aggressiveness

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Morphological features, such as tumor buddings (TBs), poorly differentiated clusters (PDCs) and micropapillary structures (MIPs), are established negative prognostic markers of aggressive colorectal cancer (CRC), exhibiting cancer cell dissociation and dissemination. Cancer cells within these tumor types undergo partial or full epithelial to mesenchymal transition (EMT), enabling them to gain enhanced migratory and invasive capabilities, which contributes to increased tumor aggressiveness and metastatic potential. Existing data define CRC tumors with a mesenchymal phenotype as corresponding to the CMS4 molecular subgroup, which is characterized by the upregulation of EMT pathways, TGF-βsignaling, matrix remodeling, stromal infiltration, and poor relapse-free survival. We recently demonstrated that the splicing signature of the NF-YA gene, which encodes for the regulatory subunit of the transcription factor NF-Y, can stratify patients on the basis of CMS subtypes. Higher expression of the NF–YAI transcript variant distinguishes the CMS4 group and correlates with the expression of EMT, extracellular matrix, and cell adhesion genes.

Based on these results, the role of NF-YAI in conferring aggressiveness to MIPs was investigated. Using RNA-FISH hybridization on tissue sections derived from patients with MIPs, we found that NF-YAI shows a higher expression and a characteristic distribution, compared to colon adenocarcinomas. Cellular models overexpressing NF-YA isoforms were generated and studied in vitro. In particular, we investigated the interaction between NF-YAI-expressing cancer cells and host cells of the tumor microenvironment, specifically focusing on two key components: tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs). 2D and 3D co-culture experiments identified a key role for NF-YAI in fostering cell interactions within the tumor niche and establishing a positive metastatic feedback mechanism.

We are currently performing spatial transcriptomic analysis on samples from patients with MIPs to identify additional biomarkers for the development of new cellular models for aggressive CRC.

# Exploring the interplay between lipid droplets and mitochondria in cancer progression

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Metabolic rewiring has been identified as a requisite for both cancer initiation and progression. Moving forward from glucose and the classical Warburg effect, as well as alterations in oxidative phosphorylation, recent findings suggest that the metabolism of amino acids and lipids is also critical for tumorigenesis. Enhanced lipid storage, in fact, arises as another common characteristic in multiple cancer cells, resulting in the accumulation of lipid droplets (LDs). Cancer cells use LDs to ensure energy production and redox balance, modulate autophagy, and boost membrane synthesis, thereby limiting stress and accelerating tumor progression. In this context, the fatty acids stored in LDs can be employed for phospholipid synthesis and mitochondrial beta-oxidation in several malignant cell types. Thus, different cancer scenarios marked by LD accumulation often display concomitant alterations of the mitochondrial compartment. However, the metabolic situations where each pathway is active, the mechanisms that control the different LD functions, and especially the link between mitochondrial dysregulations and LD accumulation are poorly understood.

Here, we want to elucidate whether specific mitochondrial aberrations could lead to LD accumulation and regulate cancer development. We used a 3D tomography-based method that allows label-free imaging and thus high temporal resolution to define LD number, dimension, morphology, intracellular distribution, and movement. The same approach has been used to visualize and monitor mitochondria without staining. Our results showed that dampened oxidative phosphorylation (by pharmacologically targeting the respiratory chain) induced changes in both the number, dimension, and distribution of LDs. Moreover, specific alteration of mitochondrial morphology, by genetically targeting the pro-fission or fusion machinery, affected LD homeostasis, as well as movement. We also observed that specific defects in calcium (Ca2+) homeostasis resulted in the accumulation of LDs and the formation of aberrant LD-mitochondria contacts. These so-called peri-droplets mitochondria (PDM) display different bioenergetics, cristae organization, and dynamics compared to cytoplasmic mitochondria, and appear to be crucial in sustaining tumor progression.

SPOKE 3, TASK 4.5

# FRG2s as a novel target for cancer treatment: a proof of principle

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Reduction in size of tandemly arrayed D4Z4 macrosatellites causes changes in the chromatin organization of the 4q35 subtelomere with aberrant expression of proximal genes. This chromosomal setting was associated with facioscapulohumeral muscular dystrophy, a rare myopathy with prevalence of 1 in 20,000. Interestingly we found that D4Z4 reduced alleles have the frequency of a common polymorphism (3-5% in the general population), an observation that indicates that other factors must intervene to cause disease. Searching for these factors, we found that genotoxic agents specifically induce the expression of FRG2A, a normally silent gene. FRG2A, belongs to a novel family of long non-coding RNAs associated with heterochromatin and possess unique features as is expression is regulated by a poised promoter and is post-transcriptionally stabilized by genotoxic stress with an inverse correlation with D4Z4 size, with higher expression level when D4Z4 is reduced.
Thus, the polymorphic D4Z4 controls the expression of FRG2A and, consequently, the capability of a cell to respond to genotoxic treatments. Therefore, we hypothesized that both D4Z4 number and FRG2A-t expression might be predictors for treatments of human malignancies.
To investigate FRG2A-t function at a molecular level, we obtained a comprehensive FRG2A transcript (FRG2A-t) -DNA and -protein interactome in Hela cells treated or not with the genotoxic drug Doxorubycin. This analysis revealed that FRG2A-t was associated with centromeric satellites (49.9%) and ribosomal DNA arrays (37.3%). Interestingly, genotoxic injury induced a strong enrichment of FRG2A-t association with centromeres which increased to the 88% of the total binding sites. Moreover, this assay revealed that FRG2 interacts with proteins involved in DNA damage response like ATRX, BRCA2 and in cell growth control like mTOR. The observation of aberrations in centromere function, which cause genomic instability, a hallmark of cancer, prompted us to investigate FRG2A-t expression in cancer cells. The responsiveness of FRG2 to drugs also led us to evaluate its impact on cell proliferation in response to chemotherapeutic treatments. We observed that FRG2A-t was at high levels in Glioblastoma multiforme (GBM) derived cells U138 and T98G GBM cells and FRG2A silencing induced a significant reduction in proliferation of T98G cells. TMZ treatment which represents the most successful therapeutic approach for GBM, also induced a strong increase of FRG2A-t levels in T98G cells. Both U138 and T98G are reported to be resistant to TMZ. Thus, we tested the proliferation of these cells with the concurrent silencing of FRG2A-t and TMZ treatment. Remarkably, we observed in both cell lines a synergistic effect of TMZ treatment and FRG2A-t silencing which sensitize cells to TMZ as demonstrated by the decrease in cell growth at lower doses of TMZ in respect to TMZ alone. Many cancers share underlying mechanisms, such as genomic instability and resistance to current therapies, that might benefit from innovative approaches. Here we provide a proof of principle that D4Z4 size and FRG2A-t function should be tested on a much larger number of cancer-derived cells and that the combined treatment with FRG2 silencing strategies and chemotherapeutics might be an effective option to overcome chemoresistance.

SPOKE 2, TASK 1.3

# Onset and progression of idiopathic pulmonary fibrosis: a role for HLA molecules

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Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive lung disease of unknown cause in which alveolar and interstitial architecture is disrupted by deposition of altered extracellular matrix. These modifications lead to restrictive lung disease, interfering with both gas exchange and lung compliance and resulting in type 1 respiratory failure. However, the pathogenetic mechanisms underlying IPF onset and progression remain elusive. Several genetic variants in the Human Leukocyte Antigen (HLA) genes has been associated with several idiopathic inflammatory diseases, including IPF. Nevertheless, the role of HLA molecules in the pathogenesis of IPF is still controversial. Taking advantage of the opportunity to study a population with low genetic variability such as the Sardinian one, our objective was to clarify the influence of HLA molecules on the onset and progression of the idiopathic pulmonary fibrosis. We compared the immune-genetic and phenotypic characteristics of 103 IPF patients with varying degrees of severity of the disease and 303 healthy controls from Sardinia (Italy). The genomic DNA was extracted from peripheral blood mononuclear cells following standard methods. All samples were genotyped at high resolution for the alleles at HLA-A, -B, -C, -DR and -G loci using Next-generation sequencing (NGS) AlloSeq Tx17 (CareDx) method based on Hybrid Capture Technology and performed on the Illumina platform. The data was analyzed using the AlloSeq Assign® software (v.1.0.2). The analysis of HLA allele frequencies revealed an overlap between IPF patients and controls, with few significant differences. However, HLA allele frequencies differed in relation to the severity of IPF, resulting enriched the HLA-B*40:02:01 allele in patients with severe forms compared to controls and patients with mild disease. This allele has been associated with several pathological conditions, suggesting a possible role in the susceptibility and progression of IPF. These genetic variations in HLA alleles could affect the control of the immune system response, leading to chronic inflammation in lung tissue, one of the main pathogenetic mechanisms of IPF.

SPOKE 1, TASK 1.3

# Sparc release upon respiratory complex I impairment supports survival and migration of ovarian cancer cells

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In the last years the involvement of mitochondrial respiration has been increasingly recognized as pivotal player during tumor progression and chemoresistance. In particular, targeting respiratory Complex I (CI) has been proposed as a new therapeutic approach to hinder cancer growth. In this context, we have demonstrated that a severe CI impairment promotes a delay of tumor expansion but not its complete eradication. Indeed, over time CI-defective cancer cells survive and reactivate their proliferation capabilities, allowing us to speculate that adaptive mechanisms occur to overcome the metabolic and molecular consequences of mitochondrial impairment. This scenario might be relevant in ovarian cancer (OC), where about 85% of patients develop relapses after standard surgical and pharmacological treatments. In this study, we investigated the impact of CI ablation on OC growth, showing that the activation of the main sensor of energy stress, AMPK, may block the kinase activity of mTORC1 contributing to confine OC cells in a low proliferative status. However, upon the mitochondrial impairment, we observed a significant increase of SPARC, a matricellular protein involved in cell-cell interaction and cell survival. We found that SPARC sustained survival and PKCβ-mediated cytoskeleton remodeling of CI-impaired OC cells. The dissection of such different pathways may offer potential molecular players in synthetic lethality with CI inhibition, thus providing new synergistic strategies for cancer treatment and, in particular for OC.

# The construction of an end-to-end platform for predicting protein-protein interactions from sequences to 3D structures

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The elucidation of protein-protein interaction networks is pivotal in understanding the molecular underpinnings of a spectrum of neuropsychiatric and neurological disorders, including schizophrenia, intellectual disability, autism spectrum disorders (ASD), and Alzheimer's disease. Integrating mutation data pertinent to these diseases into protein structural analyses is fundamental for evaluating mutations' effects on protein-protein interactions, notably among various protein isoforms. This integration is critical to unraveling the molecular mechanisms driving these disorders. To handle large datasets from omics techniques, we first developed a general automatized platform, MoNvIso, to predict structural determinants of protein isoforms, which identifies the most helpful isoform for computational modeling, balancing the coverage of mutations of interest and the availability of templates to build a structural model of both the wild-type isoform and the related variants. Building upon this foundation, we devised a hierarchical protocol that synergistically combines molecular docking, molecular dynamics simulations, and clustering techniques. This integrated approach allows for a systematic examination of the repercussions of disease-associated mutations on protein-protein interaction networks. Our methodology has been applied in two critical case studies: 1. We have predicted the structure of the mouse DOCK7/DNMT1 complex, suggested by our experimental collaborators, which potentially contributes to the pathophysiology of neurodegenerative diseases through a DNMT1-dependent regulation of the proteostasis network. A list of possible vital mutations affecting the protein-protein complex formation was proposed using our structural data, and these predicted models are currently in the phase of experimental validation; 2. We are exploring the effects of mutations related to ASD on the heteropentameric human WAVE regulatory complex (WRC), a central entity in synaptic formation encompassing CYFIP2, NCKAP1, WAVE1, ABI2, and HSPC300. Cellular assays have confirmed that such mutations lead to aberrant WRC activation, and the investigation of the underlying molecular mechanism continues. Our work will pave the way to establishing an end-to-end platform for predicting protein-protein interactions from sequence to 3D structure, facilitating the identification of key hubs for treating neuropsychiatric and neurological diseases.

SPOKE 2, TASK 1.3

# Evaluation of the impact of gut microbiota and its alteration in a mouse model of atherosclerosis and identification of new potential biomarkers

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Although gut dysbiosis has been identified as one of the factors that may contribute to atherosclerosis, additional research studies are required to deeper investigate the involved biochemical mechanisms. Specifically, it has been already reported that such condition may be affected by the production of various metabolites from different organisms in the gut microbiota in both positive way (such as short-chain fatty acids like butyrate) and/or in negative way (such as lipopolysaccharides). Furthermore, it has been shown that the use of prebiotics may alter the gut microbiota and cause the production of specific metabolites, which may benefit individuals with atherosclerosis.
This study evaluated the effects of a combination of prebiotic compounds administered alone and in combination with an extract of Red Yeast Rice (RYR) on the gut microbiota in transgenic Apoe-/- mice in a model of fat diet-induced atherosclerosis. Thanks to a proteomic approach based on LC-MS/ MS analysis coupled with bioinformatics elaboration of mice samples (i.e., serum, liver and intestine at different time points), it was possible to deeply assess the biological processes and molecular functions associated to microbiota variation and to compare the differentially expressed proteins (DEPs) between pathological and wild type (WT) model in association with biochemical parameters monitoring. Additionally, it was evaluated the pathological model in the absence or presence of treatments that affect the microbiota. Moreover, the final goal was to identify, among DEPs, new potential biomarkers that may have a prognostic or diagnostic role for the disease or could represent new molecular targets for the development of personalized treatments.

SPOKE 1, TASK 3.1

# The transcription factors NFATc1 and NFATc2 control glucocorticoid resistance in pediatric T-cell acute lymphoblastic leukemia

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Resistance to Glucocorticoids (GCs) is still a limitation in the treatment of pediatric T-cell Acute Lymphoblastic Leukemia (T-ALL) patients and a well-defined poor outcome predictor. Thus, the comprehension of GC resistance underlying mechanisms could improve T-ALL patients' overall survival. Interestingly, our research group has already unveiled the LCK kinase's pivotal role in T-ALL cells' GC resistance onset, although the downstream regulated biological processes remained to be elucidated. To this end, here we focused on the LCK downstream NFAT family transcription factors. To identify the NFAT family members modulating GC resistance we performed in vitro and in vivo proliferation assays in NFATs silenced or overexpressing T-ALL cells treated with GCs. Next, transcriptome analysis in NFATc1 or NFATc2 knock down cells allowed to infer the NFATc1 or NFATc2 driven biological processes responsible for GC resistance. Moreover, by Nuclear Magnetic Resonance and Chromatin Immune Precipitation we characterized the lipidomic landscape in NFATc1 knock down cells and the NFATc1 or NFATc2 direct target genes. We demonstrated that exclusively NFATc1 or NFATc2 specific gene silencing restores GC sensitivity in T-ALL GC resistant cells, whereas their overexpression in GC sensitive cells restores the resistance. Furthermore, we revealed that NFATc1 confer GC resistance by directly regulating the transcription of cholesterol biosynthesis' genes. In agreement, exogenous cholesterol addition to NFATc1 knock down cells rebuild GC resistance, on the contrary simvastatin sensibilizes T-ALL cells to GCs. Besides, we revealed that NFATc2 sustains GC resistance by directly controlling the transcription of LRP6, a Wnt/β-catenin pathway player. Interestingly, the Wnt/β-catenin signaling activation restores GC resistance in NFATc2 knock down cells, whereas its inhibition increases GC sensitivity. Finally, we revealed that NFATc1 and NFATc2 promote GC resistance by hindering the Glucocorticoid Receptor (GR) transcriptional activity. In agreement, diagnosed pediatric GC resistant T-ALL patients display a high NFATc1-NFATc2 and a low GR transcriptional activity. Overall, the identification of NFATc1 and NFATc2 as new regulator of GC resistance through the modulation of cholesterol biosynthesis, Wnt/β-catenin signaling and GR transcriptional activity, will provide the rationale for alternative therapeutic options to overcome T-ALL GC resistance.

SPOKE 1, TASK 2.2

# Mir-30e-3p/CXCL3 axis predicts early tumor escape in Sorafenib treated HCC patients

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Curative treatment options for hepatocellular carcinoma (HCC) remain largely limited to a minority of patients identified at early disease stages. Immunotherapy and Tyrosine Kinase Inhibitors (sorafenib and lenvatinib) represent the first-line treatments in advanced cases. Despite immunotherapy has revolutionized HCC treatment, only a minority of patients show a prolonged response. The identification of biomarkers predictive of drug response or early tumor escape remains an unsolved clinical need. MicroRNAs and chemokines are pivotal players in the progression and development of drug resistance in HCC. We previously reported higher miR-30e-3p levels in non-responder patients undergoing sorafenib treatment. Here, we aimed at identifying novel miR-30e-3p targets involved in sorafenib response and, at the same time, representing biomarkers of treatment response.
Serum and tissue miR-30e-3p and CXCL3 levels were analyzed by qPCR analysis in HCC patients and DEN-HCC rats. Functional analysis and luciferase reporter assay assessed CXCL3 targeting by miR-30e-3p in HCC cell lines. ELISA assay evaluated serum CXCL3 levels in sorafenib-treated HCC patients. Statistical analysis was performed to investigate clinicopathological associations.
CXCL3 resulted upregulated in human and rat HCCs and showed a direct correlation with CXCR2 receptor and a negative one with miR-30e-3p. Functional analysis and luciferase reporter assay demonstrated CXCL3 targeting by miR-30e-3p in HCC cell lines. In the HCC rat model, higher CXCL3 tissue levels correlated with sorafenib resistance, showing a negative correlation with apoptotic markers and tumor suppressor genes and a positive one with tumor size. Before treatment, lower CXCL3 levels associated with microvascular invasion in human HCCs. At two-month follow-up, higher CXCL3 and miR-30e-3p levels were observed in blood samples of non-responder patients. Moreover, CXCL3 levels inversely correlated with days of treatment and positively correlated with neutrophil count, whereas miR-30e-3p positively correlated with alfa-fetoprotein. CXCL3 and miR-30e-3p showed a promising predictive potential at the ROC curve analysis. CXCL3 is a novel miR-30e-3p target in HCC and is involved in sorafenib resistance. If validated in larger cohorts, CXCL3 and miR-30e-3p represent promising circulating biomarkers of early tumor escape in advanced HCCs receiving sorafenib treatment.

SPOKE 4, TASK 2.2

# SESSION 1.2

# Human PDGFRα-transgenic mouse: a novel experimental model of lung fibrosis

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Platelet Derived Growth Factor Receptor α (PDGFRα) is a key signaling molecule in the development of tissue fibrosis and one of the targets of autoimmunity in systemic sclerosis (SSc). Human anti-PDGFRα antibodies cloned from memory B cells of SSc patients (HuPDGFRα mAbs) have previously shown to increase collagen gene transcription in healthy donor skin fibroblasts, and to induce fibrosis in human skin grafts in SCID mice. In order to verify if stimulation of PDGFRα by these autoantibodies can produce tissue fibrosis in vivo, we generated human PDGFRα-transgenic mice and exposed them to systemic transfer of HuPDGFRα mAbs.
Full length human PDGFRα cDNA was knocked-in into the Rosa26 locus on mouse chromosome 6. F2 heterozygous C57BL/6-hPDGFRαtransgenic mice were used to establish the colony. On day 0 mice were subcutaneously implanted, under the back skin, with ALZET mini-osmotic pumps containing stimulatory HuPDGFRα mAbs or vehicle only control. On day 28 mice were sacrificed, and lung tissue was harvested for subsequent histological and molecular analyses. High-resolution tomography of lung tissue was performed at Synchrotron Radiation (SR) source (ELETTRA, Trieste, Italy) to investigate 3D morphology and alveolar physical density distribution.
Transgenic mice were phenotypically normal, fertile, and did not display any apparent pathological features. Human PDGFRα mRNA and protein were detectable in the lung of all examined transgenic mice. Continuous subcutaneous administration of stimulatory HuPDGFRαmAbs for 28 days determined lung fibrosis, characterized by peribronchiolar/perivascular collagen fiber deposition. 2D and 3D imaging and morphometric mapping obtained by synchrotron propagation-based phase-contrast microtomography of mouse lungs revealed increased deposition of collagen fibers around lung bronchioles and vasculature, characterized by marked alveolar thickening, reduced alveolar space and augmented alveolar volume density. None of these features was observed in vehicle only control mice.
We generated a novel humanized mouse model of lung fibrosis based on the concomitant expression of human PDGFRα and injection of stimulatory anti-PDGFRα antibodies. This model may be useful to identify new therapeutic strategies for SSc.

# Testing effect modification by folic acid supplementation in the association between pregnancy exposure to PM10 and newborn's telomere length

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Exposure to air pollution during pregnancy can trigger molecular changes potentially affecting the regulation of biological and genetic processes. Various studies have highlighted the association between maternal exposure to ambient air pollutants and molecular alterations in new-borns, including telomere length (TL). Specifically, our previous research suggests that exposure to PM10 during the 15th to 20th gestational week might be linked to shortened telomeres at birth. Since TL at birth is a crucial predictor of TL later in life, understanding the factors influencing new-borns TL is essential for preventing age-related diseases through fetal programming. Folate, a vital B-vitamin, plays a crucial role in cell growth and metabolism. 400 µg/day of folic acid (FA), its synthetic form, is recommended for pregnant women from preconception until the end of the first trimester to prevent fetal neural tube defects. Previous studies have indicated a positive association between serum folate levels and TL in adults, with potential mechanisms including DNA methylation and oxidative stress. There's a suggestion of a possible link between maternal FA supplementation during pregnancy and longer TL in new-borns. This study aims to explore the potential effect modification of FA supplementation on the relationship between pregnancy PM10 exposure and TL in new-borns within the Piccolipiù birth cohort on a subset of approximately 450 mother-child pairs. This study examined subgroups of mother-child pairs based on FA supplementation status. Specifically, we categorized pairs by the cumulative dose of FA supplementation and assess the association between PM10 exposure during pregnancy windows and TL. All analyses are adjusted for pre-selected covariates, including study center, maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's sex. The mean (standard deviation) PM10 levels through pregnancy was 32.2 µg/m3 (10.0); for 17% of the children mean exposure levels exceeded the recommended threshold of 40 µg/m3. Preliminary results suggest that the association between PM10 exposure during the first trimester and TL could be modified by FA supplementation during pregnancy (p-value for heterogeneity=0.034). In detail, children whose mothers are in the upper tertile of FA supplementation have a positive association between PM10 exposure and TL, while children whose mothers are in the lower tertile have negative associations ($\beta$ =0,0037 p=0.05; $\beta$=-0,0025 p=0,1259 respectively). Low doses of FA during pregnancy increase the negative effect on TL of PM10 exposure especially if it occurs during the first part of pregnancy. For high doses of FA, PM10 exposure increases the TL. Positive association between TL and recent exposure to air pollution have been observed in adults. It is known that disruption in the TL maintenance system conveys some sort of risk: shorter telomeres increase the risk for many age-related diseases, while longer telomeres increase the risk for some types of cancer. Identifying whether FA supplementation mitigates PM10's adverse effects on TL could have implications for public health strategies. While reducing air pollution through policy changes is vital, optimizing FA supplementation during pregnancy might yield faster results. Exploring various FA doses and durations would provide insights into potential adjustments to current recommendations.

# Advanced human respiratory epithelial cell models for delivery of repurposed anti-inflammatory agents by Nano-into-Micro delivery systems in cystic fibrosis

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To investigate the potential of smart drug delivery systems for precision medicine, our aim was to develop suitable culture models mimicking in vivo human airway epithelia to translate results into patients with cystic fibrosis (CF) to dampen lung inflammation. We have identified nasal epithelial cells (NEC), grown at Air-Liquid Interface (ALI) culture conditions, as a suitable model obtained from nasal brushings obtained from CF patients. Moreover, we have designed polymeric nanoparticles to deliver to NEC the prostacyclin analogue Iloprost, a drug already used in the clinics for treating pulmonary artery hypertension; these resulted mucus penetrating, cytocompatible and able to downregulate the expression of inflammatory genes. To be locally administered into the lungs, the Iloprost-loaded NPs were embedded into mannitol matrices, obtaining the inhalable Nano-into-Micro (NiM) microparticles.
To date, nasal epithelial brushings from 5 CF individuals (homozygous or compound heterozygous for the F508del mutation) were collected and isolated cells were expanded under conditional reprogramming culture (CRC) method, that supports the long-term expansion of airway epithelial cells with the use of RhoA kinase inhibitor. Cells from three of these expanded lines were induced to differentiate into airway epithelium, after culturing in ALI conditions, that is the "gold standard" pre-clinical model system for CF translational studies. In ALI cultures, cells are seeded onto cell culture inserts made of microporous membranes. Cells are initially submerged in cell culture medium and after reaching confluency, they are differentiated through apical exposure to air.
Differentiated NEC ALI will be analyzed for CFTR, tight junctions, mucus and cell polarity, all aspects that recapitulate the in vivo multicellular complexity. Concerning the evaluation of Iloprost-loaded NPs embedded into NiMs, differentiated NEC ALI treated with different formulations (i.e. surface pegylated or unpegylated NPs embedded into NiMs) will be analyzed by cytofluorimetry in the presence of inhibitors of endocytosis and macropinocytosis for the uptake, and then, will be studied for the modulation of inflammatory pathways by evaluating in Real-time PCR the expression of inflammatory cytokine mRNA expression levels.
Biomarker identification was carried out and up-to-date literature revealed that microRNAs (miRNAs) and exosomes were the most promising in the CF context. A preliminary evaluation of miRNAs expression was done in human CF immortalized bronchial epithelial cell line, CFBE41o-. We analyzed by Real-Time PCR the following target miRNAs (miR-145, -223, -138, -146a, -17, -155). Results show that miR-17 was expressed at high levels; miR-146a at low levels; and miR-138, miR-145, and miR-223 at very low levels; miR-155 was not expressed. Furthermore, the evaluation of exosomes size distribution from CFBE41o- cells by nuclear magnetic resonance (NMR) diffusion ordered spectroscopy (DOSY) measurements was planned. The results obtained in the context of miRNA and exosomes evaluation will be translated in primary cell cultures from CF patients in order to potential biomarkers useful for the assessment of disease progression and for therapeutic applications.

SPOKE 6, TASK 4.1

# Nose-on-chip: a 3D microfluidic platform to understand and treat sinonasal cancers

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Sinonasal cancers (SNCs) are rare and variegated head and neck cancers, for which personalized treatment approaches are still a challenge. Conventional in vitro cell cultures provide bidimensional (2D) systems that are unable to reproduce the stimuli perceived by cells in native tissues. Growing cells on three-dimensional (3D) porous biomaterial-based scaffolds and providing proper stimuli, can help to study cancer biology, test drugs, and assess therapies in a better reliable way. Among these, organ-on-chips (OOCs) provide miniaturized and finely controlled 3D dynamic systems, which can be observed in real-time.
In the framework of HEAL ITALY project, we are developing an OOC platform provided with SNC cells isolated from patients grown on 3D scaffolds resembling the nasal mucosa. Our system was designed based on the results obtained by Computational Fluid Dynamics (CFD) simulations to model the NOC flow pattern and determine the optimal flow rate to achieve a timely and uniform distribution/elimination of biological substances, and finally fabricated in poly(dimethyl siloxane) using casting and stereolithography, which was named nose-on-chip (NOC).
As a first step, 3D porous sponges based on poly(vinyl alcohol) (PVA) and gelatin (G) were produced, on which primary cell lines derived from intestinal-type adenocarcinoma (ITAC) patients were used to assess new drugs, by comparing their effect towards human dermal keratinocytes (HaCaT cells) used as non-cancerous (i.e., normal) control. Two different drug categories were tested: 1) lactate dehydrogenase (LDH) pump inhibitors: NHI-1, PI-147 and NHI-Glc-2, and 2) glucose transporter (GLUT) inhibitors: PGL-13 and PGL-14. In the static 3D in vitro model, NHI -Glc-2 had the highest action on ITAC cells, without significantly affecting the normal controls. Construct vitality, assessed through metabolic activity assay (AlamarBlue) and double-stranded (ds)-DNA content (PicoGreen assay), showed a significant viability reduction ($p < 0.001$) and a significant decrease in ds-DNA content ($p < 0.01$), compared to the action of the other drugs. Histological analysis confirmed the obtained results. In dynamic studies, the 3D in vitro model (PVA/G 90/10 (w/w) seeded with ITAC cells) was placed in the NOC device, connected to a syringe pump for continuous nutrient perfusion to the cells. CFD analysis showed that a flow rate of 0.24 µl/min provided adequate turnover of nutrients and waste within the 3D system. In this platform, an optimal cell viability was measured compared to that of static cultures.
The results obtained so far confirmed the strong efficacy of the molecule NHI -Glc-2 against ITAC cells. This outcome is highly encouraging as it suggests a targeted action of the drug against tumor cells without significantly affecting normal cells. NOC culture highlighted that ITAC cells, exposed to a slow and steady flow, maintained a high viability within the microfluidic platform. This finding is instructive on the use of a dynamic 3D culture system, such as NOC, to assess potential therapies. 3D in vitro cultures and OOC technology are useful tools for drug testing and personalized medicine for SNCs. Additionally, by using CFD simulations, native tissue conditions could be easily reproduced for realistic therapy studies.

SPOKE 3, TASK 4.1

# Gastroesophageal circulating tumor cell crosstalk with peripheral immune system guides CTC survival and proliferation

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Tumor dissemination is a key event in tumor progression. During this event a main role is played by circulating tumor cells (CTCs), immune cells, and their interaction. How the immune system supports the survival and proliferation of CTCs is not fully elucidated. In this study we describe the use of an in-vitro co-culture system consisting of immune cells and CTCs from the same patient, to increase the success rate in the establishment of CTC-derived long-term cell cultures. In this system, we characterized the immune cells of successful co-cultures and the signals they exchange with cancer cells, including cytokines and extracellular vesicle (EV) content. Using this protocol, we stabilized four CTC-derived cell lines from patients with metastatic gastroesophageal cancer, which were cultured for over a year and characterized from a genetic and molecular point of view. The four cell lines harbor shared chromosomal aberrations including the amplification at 8q24.21 containing MYC and deletion 9p21.3 containing CDKN2A/B and the IFN type I cluster. Moreover, the transcriptomic profile of CTC cell lines is distinct from primary tumors, and we detected the activation of E2F, G2M, MYC pathways and the downregulation of gamma interferon response pathway. Each cell line shows a degree of invasiveness in zebrafish in-vivo, and the most invasive ones share the same mutation in RBA14 gene. In addition, the four cell lines secrete cell-line specific EVs containing microRNAs that target YAP, BRG1-AKT1, TCF8-HDAC pathways. Overall, we highlight how the immune system plays a key role in the proliferation of CTCs through EV signaling, and how CTC cell lines genomic and transcriptomic alterations make these cells less visible from the immune system and likely responsible for the survival advantage in sites far from the microenvironment of origin.

SPOKE 1, TASK 2.2

# Mouse models of metabolism and inflammation: the role of p63 C-terminal domains

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p63 is a well-established master regulator of epithelial organization and homeostasis. In the past two decades, myriad of p63 targets and downstream pathways have been identified. Biochemically, N-terminal and core domains of p63 mediate its binding to DNA and tune its transcriptional activity. However, the role of C-terminal domains under physiological conditions in vivo remains elusive. Here, we establish an animal model by deletion of exon 13 of Trp63 (Δ13/Δ13) which codifies for C-terminal SAM and TID domains. This p63 variant resembles a natural p63Δ isoform lacking C-terminus. The excision of exon 13 by Cre is controlled by Krt14 promoter and therefore occurs only in Krt14+ epithelia such as skin, esophagus and forestomach. We demonstrate that the Δ13/Δ13 mice show growth abnormalities and die within 1-2 months. Δ13/Δ13 mice consume food and liquid similar to wild-type littermates, yet Δ13/Δ13 mice show an increased energy expenditure. At organ level, these mice show abnormalities of squamous epithelia like skin and esophagus and increased inflammation in intestine. Moreover, we observed reduced lipid droplets and smaller skeletal muscle fibers. Mechanistically, by performing global transcriptomics of skin, we showed that loss of C-terminal domains of p63 in epidermis leads an activation of IL1b-pathway suggesting a systemic inflammation. These findings highlight an important role for p63 C-terminal domains to maintain balance within epithelia.

# CNF1 toxin from E.coli induces ROS-dependent DNA damage and intestinal permeabilization: implications for colorectal carcinogenesis

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Overall, 13% of all newly diagnosed cancers are attributable to infections. In this context, bacterial toxins are emerging as promising hallmarks of colorectal cancer (CRC) pathogenesis. Recent data stemming from a previous study indicate that the CIF toxin gene from E. coli is significantly associated with pre-cancerous lesions of colon-rectum, and that toxins from E. coli as a whole have a higher incidence in adenocarcinoma patients compared to healthy individuals. Interestingly, among E. coli toxins, also the gene for CNF1 is overrepresented in CRC patients colonized by E. coli. In vitro studies carried out over the years on CNF1 have shown that the toxin induces Rho GTPases activation, multinucleation, protection from apoptosis, migration and epithelial-tomesenchymal transition. However, a definitive answer on the carcinogenic capacity of CNF1 does not exist, yet. Therefore, to study the implication of CNF1 in colorectal carcinogenesis, we investigated the possible genotoxic effect of CNF1 on intestinal epithelial cells and its ability to modulate intestinal permeability. We observed that, in normal IEC-6 epithelial cells, CNF1 induces DNA damage through the release of reactive oxidizing species (ROS). Consistently, analysis of the cell cycle showed that the toxin slows down cells through the G2/M phase. This effect, however, is overcome by CNF1 removal from culture medium. Although predominantly reversible, the CNF1-induced DNA damage is capable of inducing, in the long term, genomic instability mainly represented by aberrant chromosomal structures, chromatid breaks and polyploidy. Beyond inducing genomic instability, gut permeability disruption also plays a role in CRC aetiology. Exposure of Caco-2 monolayers to CNF1 reduced transepithelial resistance (TEER) by 1/3 as compared to non-treated monolayers. Immunofluorescence confocal microscopy analyses show that this effect correlated with a significant down-regulation of the tight junction protein ZO-1 in CNF1-treated cultures, with respect to control samples. Differentiated advanced 3D Caco-2 spheroids with a central lumen, exhibiting traits of intestinal epithelium in vivo are presently being used to confirm and further characterize CNF1-induced alterations of intestinal barrier integrity. Taken together, our results suggest that the E. coli CNF1 toxin may exert effects promoting malignant transformation, possibly concurring to colorectal carcinogenesis in vivo.

SPOKE 7, TASK 1.1.2

# SESSION 2.1

# Designing a hybrid AI platform compliant with the European AI act: an MLOps perspective

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With the recent approval of the AI Act by the European Parliament, there is a pressing need to develop artificial intelligence infrastructures that not only meet safety and transparency requirements but are also capable of adapting to an evolving regulatory framework. This study outlines the design of a hybrid AI platform that leverages Kubernetes orchestration to manage distributed workloads both on-premises and in the cloud. This platform is optimized for low-latency operations at the edge and computationally intensive tasks in the cloud. Our architecture employs open-source components for managing the lifecycle of AI models, complemented by proprietary tools for advanced monitoring and model evaluation. This integration ensures compliance with the regulations set forth by the AI Act, providing traceability, auditability, and maintenance of AI model performance.
The discussion emphasizes the importance of building resilient and regulatory-compliant AI infrastructures, offering practical insights on cutting-edge tools and techniques in the field of MLOps. This topic is particularly relevant for professionals working in the artificial intelligence sector, striving to maintain a balance between technological innovation and regulatory compliance, critical aspects in the context of the HEAL-ITALIA precision medicine project.

# AI-driven transcriptomic encoders: from explainable models to accurate, sample-independent cancer diagnostics

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In the rapidly evolving domain of medical technology, the use of sophisticated algorithms for deciphering transcriptional data has emerged as a critical aspect, especially in the oncology sector. These algorithms, drawing upon methodologies from fields such as natural language processing and advanced image analysis, are significantly enhancing the accuracy in predicting cancer-related molecular states. Notably, Transformer models, renowned for their proficiency in handling extensive datasets, are now being innovatively adapted for breakthroughs in medical diagnostics or in stratifying patients according to prognostic levels.
This study enhances the field of precision medicine by integrating Transformer-based learning, exemplified by the Geneformer model, with Explainable AI techniques. These techniques are employed to discern which input variables (genes resulting from genomic transcription) are most correlated with the decisions of neural systems. This insight, a key goal in genomic research, aims to select the most relevant gene subset for each specific task to which a neural network is applied. This selection approach is effective in two classification tasks, cell type classification and breast cancer type classification, even across various cohorts of patients. When applying Geneformer-like architecture analyses solely to the selected gene subsets, the outcomes either maintain their accuracy or show significant improvement. This approach not only aims to contribute to the identification of vital genetic markers in cancer genomics, but also to exemplify the adaptability of AI models to different datasets in order to mark a significant step in the development of accurate and universally applicable diagnostic tools.

# AI for lung cancer imaging: a FAIRness story

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Lung cancer is the leading cause of cancer-related deaths globally for both men and women. Despite large trials demonstrating that low dose computed tomography (LDCT) can reduce lung cancer mortality by over 20%, its efficacy is hampered by a high false positive rate. Given its ability to identify hidden patterns, researchers are investigating the use of artificial intelligence (AI) tools in lung cancer screening as a solution. However, current studies often fail to meet the transparency and reproducibility specifications required to bring AI research into clinical practice.

To address this gap, we developed a framework for standardize both lung cancer screening imaging data and AI-based tools. The standard we opted in data storage is the Brain Imaging Data Structure (BIDS): establishing both the files format and the folders names and structure makes a dataset more human and machine-readable. Using BIDScoin, an open-source conversion tool, we successfully converted nearly 5 million LDCT images (n=4,924 subjects) from the National Lung Screening Trial from DICOM to BIDS. Since BIDS was originally designed for neuroimaging and does not support computed tomography (CT) modality, we are collaborating with the leader of the extension proposal to formalize its extension for CT.

For the AI application, we selected Sybil (Mikhael et al., JCO 2023), an AI model developed at MIT that predicts lung cancer risk 1 to 6 years after the LDCT exam. The work could not be exactly reproduced because only the source code (and not its dependences) was available; hence, we wrapped Sybil in a container. We used the container structure proposed by BIDS-App since it ensures that the obtained Docker and Singularity containers accept BIDS data as input and produce BIDS data as output. This standardization provides a reproducible and interoperable tool that can be run independently of the operating system and other dependencies. The final data and app standardization required 4 and 6 days, respectively, excluding the early exploration and adaptation phases.

This approach, in which data and applications are made FAIR (Findable, Accessible, Interoperable, and Reproducible) concurrently, not only streamlines the development framework but also guarantees that the research is conducted with scientific rigor, ensuring integrity and robustness. By adhering to these principles, our framework can significantly enhance the reliability and validity of AI tools in lung cancer screening and pave the way to their clinical implementation, improving screening outcomes. Furthermore, it fosters greater collaboration and data sharing among researchers, accelerating advancements in the field and promoting the widespread adoption of AI in clinical settings.

Building on this foundation, we aim to further validate our framework in larger, diverse populations and explore additional AI models for integration.

SPOKE 2, TASK 3.3

# MedBooster: a general framework for designing novel self-supervised learning paradigms tailored for the medical domain

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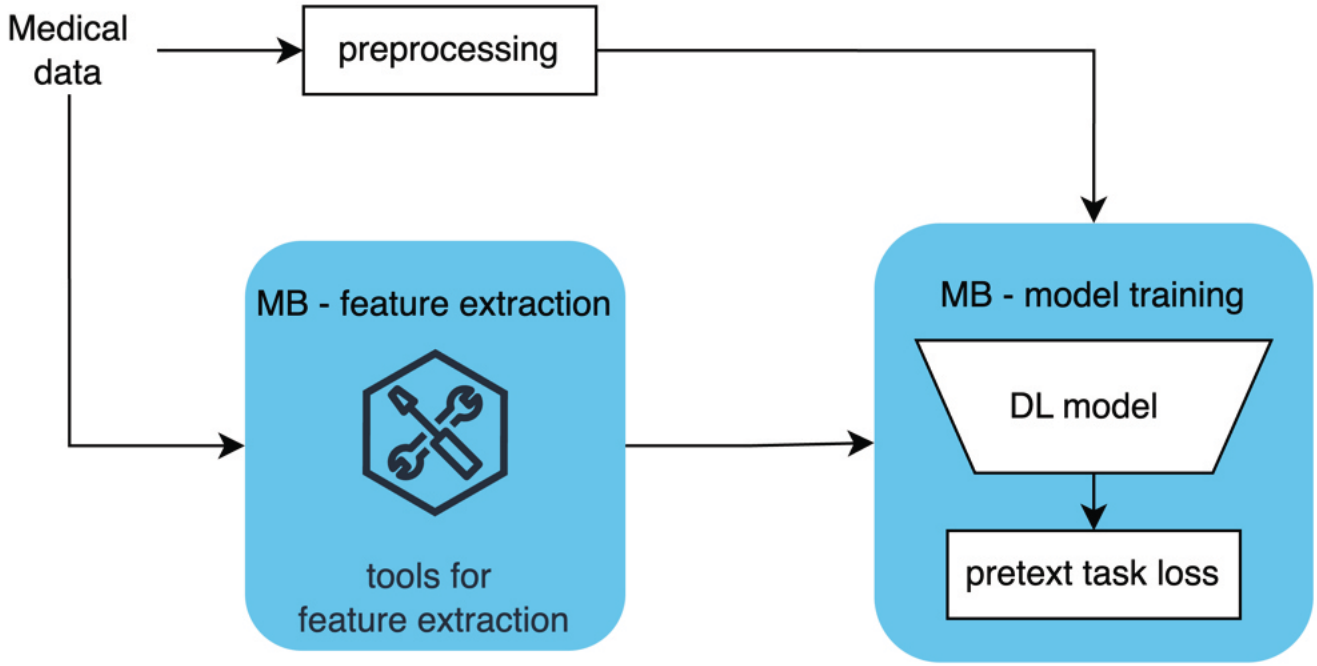
In the realm of medical research, the adoption of Self-supervised Learning (SSL) methodologies has shown promise in reducing dependence on labeled data while enhancing generalization capabilities beyond conventional supervised learning (SL) approaches. Despite these advancements, current SSL techniques are primarily developed for generic domains and are typically evaluated using standard datasets such as ImageNet. Recent investigations have highlighted the superiority of SSL methods customized for medical applications over domainagnostic counterparts. This study introduces MedBooster, an innovative framework designed to facilitate the creation of specialized SSL methodologies tailored for diverse medical contexts. At its core, MedBooster leverages automated feature extraction tools to construct pretext tasks, thereby advancing SSL performance in medical scenarios. In this study, we implement and experiment with MedBooster for brain magnetic resonance imaging (MRI) analysis. The pretext task involves the regression of brain MRI features extracted from imaging data. To assess MedBooster efficacy, we conducted comparative analyses against traditional SL techniques and two leading domainagnostic SSL paradigms. Our evaluations encompassed a regression task (age prediction) and a classification task (distinguishing patients with Alzheimer's disease from cognitively normal individuals). Each pretraining and fine-tuning experiment was repeated across 30 randomized seeds to estimate the uncertainty of the results. Statistical analysis of the results reveals MedBooster superiority across various scenarios. Notably, even with only 1% of labeled data available, MedBooster achieves a significantly lower average Mean Absolute Error (MAE) of 15.00 years compared to SL's second-best average score of 20.67 years. These findings show MedBooster efficacy and potential, suggesting MedBooster utility as a roadmap for crafting specialized SSL methodologies tailored specifically for medical research endeavors. The figure below shows the MedBooster schema.




SPOKE 2, TASK 2.1

# Generative models for medical imaging

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Deep learning (DL) demonstrates significant potential in medical imaging. However, its application in precision medicine is limited by data scarcity, largely due to privacy concerns. One strategy to address this issue is data augmentation through basic or deformable techniques. However, these methods are bound by the limit of generating highly correlated data with the original dataset. An alternative approach involves using generative models to produce synthetic data, thus expanding the dataset size or creating entirely synthetic datasets with reduced privacy concerns. Generative models such as generative adversarial networks (GANs) and diffusion models, can learn the underlying data distribution from real datasets and exploit this knowledge to generate new, realistic data adhering to the same distribution. Several studies have already demonstrated the potential of GANs to improve classification performance when artificially growing the training dataset with synthetic data. Since GANs are obtained through the training of DL models, they still depend on the size of the training dataset.

In our experiments, we used StyleGAN2-ADA, a state-of-the-art GAN proposed by Karras et al. from NVIDIA, designed to generate realistic synthetic images when working with small training datasets. To assess the synthetic images qualitatively, we asked a radiologist with over 30 years of experience to perform a visual Turing test. We presented 2000 brain magnetic resonance (MR) images (1000 real and 1000 synthetic) in random order, using a custom application that allowed window/level adjustment. The radiologist could not reliably distinguish real and synthetic images, indicating the anatomical realism of the synthetic images.

However, generative models can still exhibit failures, which necessitates quantitative evaluation. While GANs are less prone to overfitting than diffusion models, they can still overfit and suffer from mode collapse, where only part of the data distribution is learned. Specific metrics are required to investigate these issues. The Fréchet Inception Distance (FID) and Kernel Inception Distance (KID) are commonly used to evaluate the similarity between real and synthetic data distributions. However, these metrics do not explicitly examine mode collapse and overfitting.

Mode collapse can be assessed using precision and recall (P&R), which quantifies the fraction of realistic generated images and the fraction of the training data distribution covered by the generative model, respectively. Enhanced metrics like $\alpha$-precision and $\beta$-recall and density and coverage (D&C) also measure these aspects. The mode collapse can also be quantified by training classifiers on synthetic data and evaluating their performance on real data, using metrics such as essential to ensure the generation of high-quality, realistic synthetic data without overfitting or mode collapse. the classifier 2-sample test, classification accuracy score (CAS), and the GAN quality index (GQI). Overfitting can be examined using the authenticity score, which measures the fraction of synthetic samples not copied from the training data. Graphical such as k-Nearest neighbor (k-NN) analysis and t-SNE visualization can also aid in assessing overfitting.

In conclusion, while generative models like StyleGAN2-ADA show promise for augmenting medical imaging datasets, thorough evaluation using a combination of qualitative and quantitative metrics is essential to ensure the generation of high-quality, realistic synthetic data without overfitting or mode collapse.

# Quantum extreme learning machine for protein classification

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Nowadays, machine learning (ML) stands as a fundamental tool to analyze vast datasets, in order either to classify them or to recognize complex patterns. Recent strides suggest that the integration of quantum mechanics (QM) phenomena with ML algorithms holds tremendous potential for enhancing their performance. This synergy has birthed a new frontier known as quantum machine learning (QML), where emerging evidence suggests quantum advantages in processing classical data. While initial validations of QML algorithms have been conducted on modest datasets via simulations on classical hardware, their favorable scalability promises the handling of larger datasets and more intricate tasks. Yet, practical implementation for applications of industrial or pharmacological interest remains a challenge, primarily due to the prevalent noise in current quantum hardware.

Among the array of QML algorithms, the quantum reservoir computing class, and in particular quantum extreme learning machine (QELM), emerges as one of the most ambitious paradigms. This routine, operating within the realm of supervised learning, endeavors to discern intricate patterns that map input data (e.g., structural information of a protein such as its primary sequence) to corresponding output variables (e.g., specific protein properties we aim to predict). Here, the input data can be encoded into the quantum states of 'qubits' and processed through quantum processors. Physical measurements of these qubits enable the extraction of classical information, which is then refined to approximate the desired complex patterns. QELM, adept at minimizing quantum resource utilization, delegates much of the training workload to classical computers.

We apply the QELM paradigm to a binary classification task involving proteins. The dataset comprises a unique selection of proteins or peptides, characterized by a specific set of descriptors experimentally validated, with the aim of categorizing them into binary outcomes, such as hemolytic or non-hemolytic. The relatively high number of input features to describe the proteins, 40 different descriptors, constitute a benchmark for the state of art of QELM. Our findings suggest that QELM may outperform the respective classical ELM. Moreover, by implementing the algorithm on quantum EAGLE processors by IBM, we ascertain its resilience against noise, heralding its potential for imminent applications at an industrial pharmacological scale.

# A key performance indicator to analyze swarm learning performances with EHR

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Swarm Learning (SL) has been recently proposed for distributed learning, where a group of individual centers perform synchronized training. Unlike traditional machine learning models that rely on a central server, swarm learning distributes the learning process across multiple nodes. Each node independently processes data and contributes to the overall learning task. This collaboration allows the swarm to benefit from individual nodes' different data. Unlike federated learning, here, model parameters are not handled by a central server but are randomly handled across each individual node. The intrinsic attention of swarm learning to data privacy makes it suitable for distributed healthcare analysis, where a clinical center wants to benefit from all the other ones in the swarm network. However, the benefit for a single center or the whole network could vary depending on data distribution. Here, we want to analyze the performance of the swarm learning in a network with multiple nodes, where different data distribution scenarios are considered.

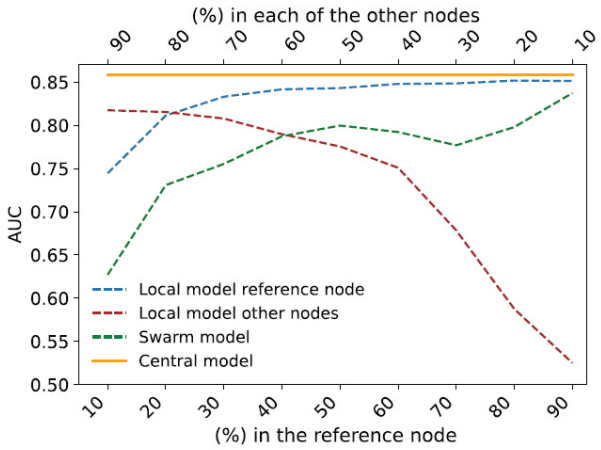


*Figure 1 - Average AUC performance of the models with different data distribution scenarios. In particular, the figure shows the models for the reference node, the other nodes, the swarm network, and the central one.*

This analysis will show the gain of the whole swarm network and a specific (reference) node, focusing on scenarios where this node has a different amount of data concerning the other nodes. We performed the model's training in the reference node with a growing amount of the whole training set and equally distributed the remaining percentage of the training set to the other nodes. In particular, we investigated the behavior of the swarm learning framework by running multiple experiments for the same distribution to assess an average performance, namely the Area Under the ROC curve (AUC) (see Figure 1). We computed the performance of the models using the same test set for all the experiments to compare models and the swarm model consistently. Moreover, we introduced a new key performance indicator (KPI) to measure the whole gain for the swarm network depending on the data distribution between the nodes. We applied this method using intensive care unit (ICU) data extracted from the MIMIC electronic health record (EHR) database and discussed the results obtained by analyzing different data distribution scenarios.

# Alpha&ESMhFolds: a web server for the comparison of 42,942 AlphaFold2 and ESMFold models of the human reference proteome

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Results from CASP15 confirm the relevance of Artificial Intelligence-based modelling on the accuracy of protein structure prediction. The best performances are reported by methods differently based on DeepMind's AlphaFold2. As an alternative, methods such as ESMFold take advantage of Protein Language Models, allowing for faster computations. To compare the two approaches, we develop a novel database storing AlphaFold2 and ESMFold models for 42,942 proteins covering the Human Reference Proteome, alongside 2,900 experimental structures from the PDB.

Proteins adopted in this study come from the human Reference Proteome. From the initial set, we exclude fragments, short peptides, and sequences for which we failed to obtain a model, retrieving 42,942 protein sequences. We then extract structural data from the PDB. We exclude all structures with coverage <70% to the UniProt sequence, retaining the best PDB for 2,900 proteins. Both methods compute for each residue a pLDDT value (ranging from 0 to 100) that provides an estimate of the quality of the prediction. We evaluate the quality of each model by computing the percentage of residues with pLDDT ≥70. Additionally, to evaluate the structural similarity, we adopt Foldseek to superimpose paired models and to superimpose each model to the associated PDB structure. This produces a TM-score (ranging from 0 to 1) for each superimposition.

When comparing the TM-scores of AlphaFold2 and ESMFold models against the PDB structures, for 81% of the dataset the difference is <0.1, showing similar performances for the two methods. For the remaining proteins, AlphaFold2 tends to perform better than ESMFold. This is expected, as the first method retrieves known templates during the prediction phase. Looking at the whole dataset, we find that for 45% of the proteins the TM-score between the two models is >0.6. The remaining 23,701 proteins are predicted with diverging models endowed with TM-scores <0.6. Focusing on the latter subset, we compare the quality of the paired models, observing that the more the two models diverge (at decreasing TM-score), the more the confidence of each model decreases. Moreover, AlphaFold2 seems to generate better quality models than ESMFold for 62% of the proteins. Being this a self-predicted assessment of the model quality, the value is however not sufficient for an estimate of the actual model validity.

Our database is accessible as a web server at https://alpha-esmhfolds.biocomp.unibo.it/, allowing users to easily compare models for 42,942 human proteins. The resource offers different search criteria. For all entries of the database, it is possible to visualize multiple information including general protein data, the structural superimposition and the sequence alignment between the two models and, when available, between each model and the PDB chain.

Alpha&ESMhFolds supports the analysis of human proteins by comparing high-quality models developed with 2 different methods based on artificial intelligence. This is especially relevant in the absence of an experimentally resolved 3D structure. The predicted models can also aid in the characterization of residues that can be interested by mutation, possibly hampering the stability and the function of the protein, eventually leading to the onset of diseases.

# Exposome evaluation as model for asbestos-related disease prevention a pilot study

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Although asbestos has been severely restricted or entirely ban asbestos use in many countries, still 125 million people worldwide are at risk from occupational/environmental exposure to asbestos. The population that has been professionally exposed to asbestos is decreasing. However, asbestos present in old buildings and in other types of constructions, and also in the natural environment will continue to cause exposure and may cause pleural mesothelioma (PM). Asbestos is considered the carcinogen mostly involved in the development of PM, however, only 5% of people exposed to asbestos develop the disease. The carcinogenesis is the result of a complex interactions between exogenous factors (environmental or occupational) and endogenous processes modulated by the individual genetic phenotype. Exposome provides a new approach for conceptualizing the roles and relationships of multiple exposures in the etiology and progression of cancer, over the life course and across generations. In the present study, by using environmental sensors, geographic information systems (GIS), and personalized questionnaire, external exposure (general and specific) was estimated in subjects previously exposed to asbestos with and without benign or malignant asbestos-related diseases. Therefore, a population of asbestos-exposed subjects without asbestos-related diseases (ARDs)(Exp)(males, age 66.0 ± 13.0 years), asbestos-exposed subjects with ARDs (Exp-ARDs)(males, age 67.0 ± 8.5 years), patients with pleural mesothelioma (PM)(males, 75.6 ± 8.5 years) and non-related asbestos exposure cancers (Other)(males, 69.5 ± 13.3 years) were enrolled at the clinic of occupational medicine, AOU of Marche, Ancona, Italy, and evaluated for external general exposome (distance from industrial area, shipyards, airport, port, refinery, landfill, railway line, highway, and number of pollution sites), and external specific exposome (age, BMI, smoking, education, working and asbestos-exposure duration). Among the variables included in the general external exposome, the incidence of asbestos-related cancer (PM) was higher around the refinery area, while the number of pollution sites, port, and refinery area mainly affected the incidence of cancer non-related to asbestos exposure (prostate, bladder, renal cancer). The evaluation of specific external exposome revealed that age, smoking, and the years of asbestos exposure were involved in the development of PM (Figure). The strong link between carcinogens, such as asbestos, and exposure to environmental pollution highlight the need for a more "One Health approach" in epidemiology. Knowledge about cancer biological mechanisms, exposures, and genetic susceptibility way well provide opportunities to develop precision prevention and early detection strategies.

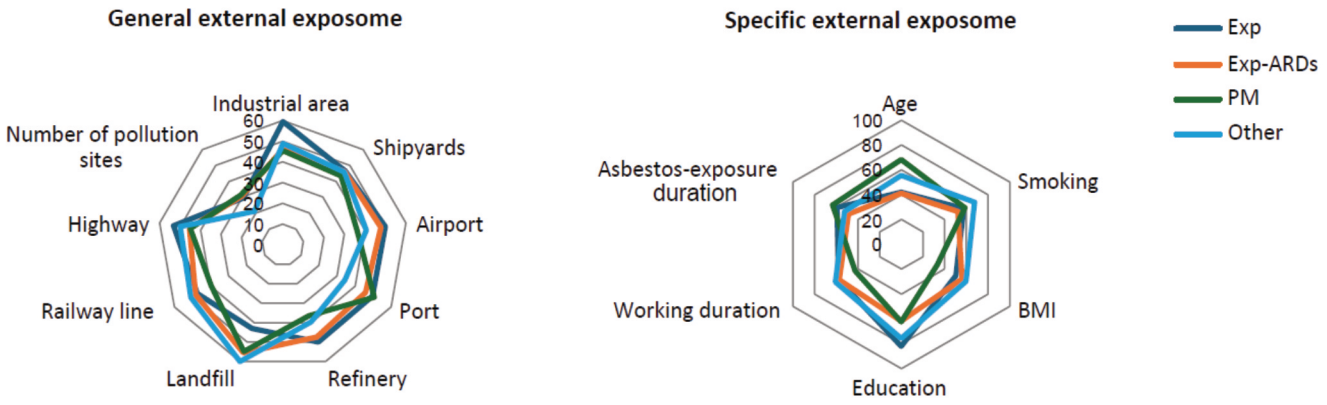


**Figure:** general and specific external exposome

SPOKE 7, TASK 1.2

# Correlation-based network integration of lung RNA sequencing and DNA methylation data in chronic obstructive pulmonary disease

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*[1] Department of Computer, Control and Management Engineering, Sapienza University of Rome, Italy; [2] Department of Experimental Medicine, Sapienza University of Rome, Italy; [3] Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.*

Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous, chronic inflammatory process of the lungs and, like other complex diseases, is caused by both genetic and environmental factors. Detailed understanding of the molecular mechanisms of complex diseases requires the study of the interplay among different biomolecular layers, and thus the integration of different omics data types. In this study, we investigated COPD-associated molecular mechanisms through a correlation-based network integration of lung tissue RNA-seq and DNA methylation data of COPD cases (n=446) and controls (n=346) derived from the Lung Tissue Research Consortium. First, we performed a network-based analysis to build separate correlation networks for RNA-seq and DNA methylation data for our case-control study population. Then, we developed a pipeline to integrate the results into a coupled network of differentially expressed and differentially methylated genes to investigate their relationships across both molecular layers. The functional enrichment analysis of the nodes of the coupled network revealed a strikingly significant enrichment in Immune System components, both innate and adaptive, as well as immune-system component communication (interleukin and cytokine-cytokine signaling). Our analysis allowed us to reveal novel putative COPD-associated genes and to analyze their relationships, both at the transcriptomics and epigenomics levels, thus contributing to an improved understanding of COPD pathogenesis.

SPOKE 2, TASK 2.2 and 3.2

# Innovative computational methodologies and platforms for drug repositioning and screening

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*University of Modena and Reggio Emilia, Modena, Italy*

Drug repurposing (DR) is a strategy that allows the identification of novel therapeutic indications for drugs, as well as synthetic and natural products (Langedijk, J. et al. DOI: 10.1016/j.drudis.2015.05.001). In recent years, drug repurposing has emerged as a promising alternative or complement to traditional drug discovery, particularly when performed using computational methods. In silico approaches, such as chemoinformatics and artificial intelligence (AI), capable of mining and analyzing vast amounts of structural, chemical, biological, and clinical information from repositories, have become central to drug repurposing, especially when integrated into computational platforms (e.g. LigAdvisor - https://ligadvisor.unimore.it) and tailored workflows (Figure 1).
In line with the main objectives of SPOKE 5, we report the results of our studies aimed at defining putative drugs and therapeutic targets via computational tools. A computational platform based on integration of AI, chemoinformatics and structure-based methods will be presented.
Applications of the platform in the field of virtual screening of drug libraries against cancer and neurodegenerative diseases will be discussed. Among these, we present the results of investigations conducted through tailored in silico workflows including ligand-based similarity estimations and classification AI-based algorithms for the repositioning of already marketed drugs and preclinical candidates against prostate cancer and gastric tumors. Moreover, we describe our recent findings related to the identification of a polypharmacological drug candidate that restores the physiological Tau-microtubule interaction in neurons, for the treatment of Alzheimer's disease. The compound was identified from a virtual screening campaign that included in silico analyses on reported aggregation inhibitors and cryo-EM 3D structures of Tau (Pinzi et al. DOI:10.3390/molecules26165039, Pinzi, L. & Bisi, N., et al. DOI:10.3390/molecules28114544), followed by extensive experimental testing through live-cell imaging assays. Altogether, the results provide further confirmation of how innovative computational methodologies and platforms can support and accelerate drug discovery.

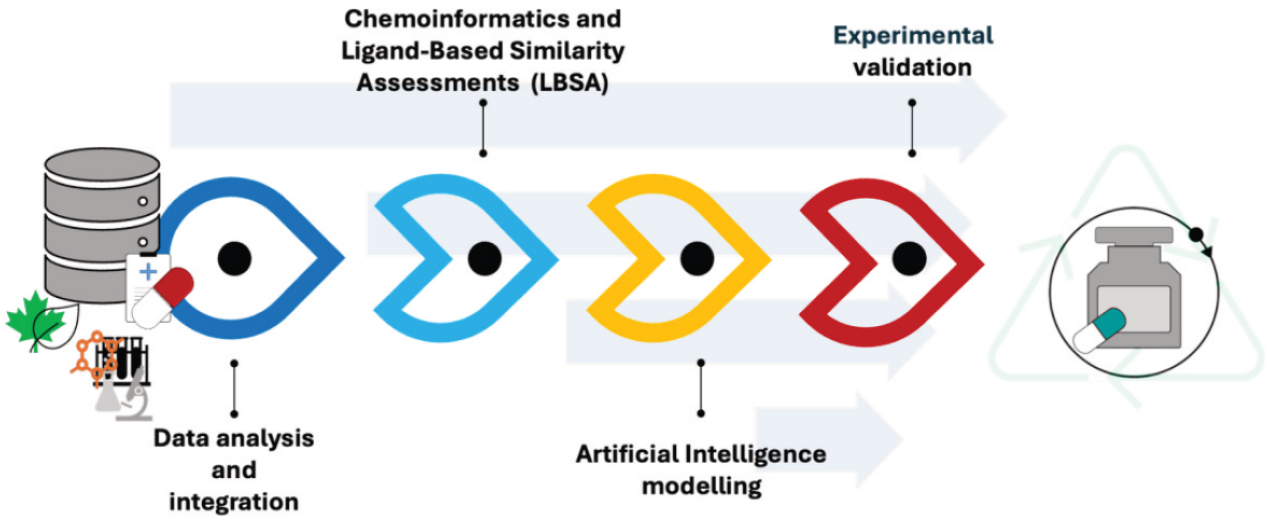


**Figure 1**: Example of *in silico* drug repurposing integrated approach.

# Predictive modeling and experimental control of macrophage pro-inflammatory dynamics

J. Riccio [1], L. Presotto [1], L.Doniza [2], D. Inverso [3], U. Nevo [2], G. Chirico [1]

*[1] Università degli Studi di Milano-Bicocca, Milano, Italy; [2] Tel Aviv University, Tel Aviv, Israel; [3] Vita-Salute San Raffaele University, Milan, Italy.*

Macrophages are innate immune cells which play a key role in tissue repair and regeneration, as well as in the inflammatory response to implanted biomaterials. These cells exhibit a functional phenotype based on the stimulus received and surrounding microenvironment. The process, referred to as polarization, is regulated by specific cytokines, which are both released by the macrophage itself and produced by other cellular activation mechanisms. Depending on their role in the immune response, whether they promote or limit inflammation, cytokines can be classified as either pro-inflammatory or anti-inflammatory, respectively. They act as phenotype markers for macrophages within a heterogeneous range whose extremes are identified as pro-inflammatory or M1 and anti-inflammatory or M2. This project is aimed at proposing a predictive modeling approach for the simulation of the response over time of the cell populations involved in a foreign body reaction (limiting, at this stage, to macrophages, fibroblasts and endothelial cells), after the implantation of a biomaterial, simulated as a mechanical stimulus. This is the basis for the development of a digital twin of reaction to biomaterials in small animal models, as well as of the inflammatory response in the atrium, through the cross-talk among atrial cardiomyocytes, immune cells and fibroblasts in fibrosis and atrial fibrillation. The dynamic plasticity of macrophage lends itself well to being reproduced by temporal models including Ordinary Differential Equation (ODE) models and Agent Based (AB) approaches. In this work, two existing ODE models, proposed by Maiti et al. and by Minucci et al., and one AB scheme proposed by Minucci et al., have been considered. In the ODE models, a pro- and an anti-inflammatory pathway, representing TNF-α and IL-10 cytokines, respectively, are included through feedback regulations on NF-kB factor, under constant lipopolysaccharide stimulation, to simulate single-cell kinematics. In the AB model, macrophages are modeled as mobile agents which diffuse in a bidimensional grid according to simplified rules and whose interaction is influenced by their spatial position and polarization. The model refers to adimensional state variables including, among others, pro and anti-inflammatory mediators acting as stimuli, and the levels of pro- and anti-inflammatory activation (M1 and M2 activation, respectively), which depending on their values, steer macrophage towards one phenotype rather than another. Models were validated by using three pro-inflammatory cytokines (TNF-α, IL-12, INF-γ) derived from in-vitro experiments. Preliminary results expressed as Pearson's correlation coefficients highlight a better agreement of the AB approach over the ODE models (Table 1). This specific scheme makes simplified assumptions on spatial resolution and diffusion of inflammation. However, the good agreement observed encourages the use of a more advanced hybrid platform based on AB modeling,

Table 1: Pearson's correlation coefficients $\rho$ between simulation and experimental results

| Model | Variable | $\rho$ | $p$-value |
|---|---|---|---|
| ODE (Maiti) | (TNF-$\alpha_{cyto}$,TNF-α) | -0.86 | 0.03 |
| ODE (Minucci) | (TNF-$\alpha_{cyto}$,TNF-α) | -0.16 | 0.77 |
| AB (Minucci) | ($M1_{act}$,TNF-α) | 0.87 | 0.02 |
| | ($M1_{act}$,IL-12) | 0.89 | 0.02 |
| | ($M1_{act}$,INF-γ) | **0.93** | $6.7e^{-3}$ |
| | ($M1_{count}$,TNF-α) | 0.72 | 0.11 |
| | ($M1_{count}$,IL-12) | **0.98** | $9.0e^{-4}$ |
| | ($M1_{count}$,INF-γ) | 0.85 | 0.03 |

# In silico study: Irisin and Vitamin D as potential allies in diabetes and COPD

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Diabetes, a chronic disease characterized by elevated blood glucose levels, poses a significant public health challenge worldwide. Beyond the well-known vascular and neurological complications, diabetes can profoundly impact muscle health. Hyperglycemia and insulin resistance in diabetes also can contribute to systemic inflammation and weaken respiratory muscles, worsening COPD symptoms. This leads to breathing difficulties, fatigue, and reduced quality of life. This condition results in the loss of muscle mass and strength, increasing the risk of falls, frailty, and disability. A holistic approach to managing diabetes and its muscle complications, particularly in conjunction with COPD, is crucial for improving patients' health and quality of life. Smoking cessation, a healthy diet, and regular physical activity are critical elements for enhancing pulmonary and muscle health. Vitamin D is a fat-soluble vitamin that plays an important role in skeletal muscle health. Vitamin D exerts anti-inflammatory and antioxidant properties that protect muscle cells from damage. Vitamin D deficiency exacerbates skeletal muscle dysfunction in COPD patients. Vitamin D supplementation improves muscle strength, endurance, and physical performance in patients with COPD. In skeletal muscle cells, vitamin D induces the expression of FNDC5, which is converted into irisin, a protein produced by muscles in response to physical exercise. Irisin's potential benefits include protection against various health problems such as obesity, insulin resistance, and fatty liver disease. The protective effects stem from irisin's anti-inflammatory and immune-modulating properties, regulating the expression of inflammatory molecules like TNFα and IL-1β, which are elevated in COPD. Irisin possesses also antioxidant and anti-apoptotic properties, further promoting cellular health. Our study focuses on the investigation the interplay between vitamin D and irisin in COPD patients with different Body Mass Index (BMI) and muscle dysfunction. Therefore, bioinformatic analysis was used to identify the genes regulated by vitamin D and irisin in order to identify the signaling pathways activated in these pathological states, thus expanding our knowledge of the underlying molecular mechanisms and paving the way for potential therapeutic developments.

# Diabetes and prediabetes as risk factors in pre- and post-liver transplantation

M. Scopelliti [1], G. Gezici [2], P. Carrai [3], S. Petruccelli [3], G. Catalano [3], C. Martinelli [3], J. Bronzoni [3], D. Pezzati [3], G. Tincani [3], E. Balzano [3], F. R. Femia [1], R. Del Testa [1], R. Pellungrini [2], D. Pedreschi [2], F. Giannotti [2], P. De Simone [3], D. Ghinolfi [3], L. Marselli [1], P. Marchetti [1]

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Discussion is still ongoing on the prevalence and characteristics of diabetes (DM) and prediabetes (PD) [IFG and/or IGT] in patients before and after liver transplantation (LT), and the impact on post-LT outcomes.
In this single center study we evaluated 1,468 candidates for LT [(age: 56±9 yrs; M/F: 1107/361; BMI: 24.4±5.8 Kg/m$^2$; family history of diabetes (FHD): 37%; FPG: 109±36 mg/dl; HbA1c: 35±12 mmol/mol); main indications for LT: 57.7% HCV/HBV-related cirrhosis/HCC, and 27.5% dysmetabolic/esotoxic cirrhosis/HCC)]. From this cohort, 1,086 subjects underwent LT, 470 of which reached a 5-year follow-up.
Based on pre-LT history, FPG, HbA1c and/or OGTT, 32.5% patients from the pre-LT cohort had DM and 21.4% PD. DM and PD patients were older and predominantly males (both $p<0.001$). Furthermore, DM subjects had higher BMI and FHD than PD and normoglycemic subjects (NG) (both $p<0.05$).
In the transplant cohort with 5-year follow-up, patient and graft survival was respectively 84.5% and 84.1%, and the prevalence of DM and PD increased respectively to 49% ($p<0.001$) and 29% ($p=0.053$) vs pre-LT. To identify pre-LT factors in NG associated with post-LT DM (PTDM), machine learning algorithms (both interpretable and explainable) were used (i.e. decision trees, explainable boosting machines and CatBoost) to assess multivariate correlations. Inspection of the models indicated that pre-LT FPG, BMI, smoking and eGFR were among the main factors correlated with PTDM. In addition, insulin therapy at discharge after LT was more common in NG and PD patients who later developed PTDM than in those who remained non diabetic ($p<0.001$). Long duration (more than 10 years) of pre-LT DM ($p=0.051$) and HbA1c >7% ($p=0.094$) tended to impact on patient survival and/or graft outcome.
This assessment of glycemic status of candidates for LT demonstrates a high prevalence of DM and PD (> 50%); it also identifies modifiable factors to possibly prevent PTDM; duration of pre-LT DM and its control could affect post-LT outcomes.

# Transfer learning approach to classify high-imbalance and multi-class mixed-patterns fluorescence images

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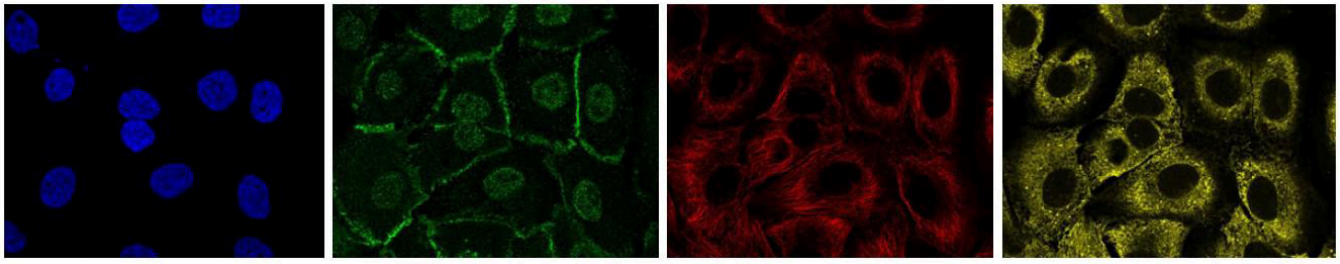
In recent years, image classification with deep learning techniques has frequently outperformed human performance in various specialized domains. However, its performance can be still low in complex fields characterized by highly imbalanced datasets and multi-class classification with mixed-pattern labels. This is the case with Human Protein Atlas (HPA) dataset, which comprises more than 42,000 samples of cells captured using confocal microscopy. The HPA is an open-access Swedish project that creates a freely available map of all human proteins in cells, tissues, and organs. The importance of automatically identifying mixed and rare protein patterns in microscope images is underscored by the competition hosted on Kaggle. The competition's goal was to develop a model with high performance that approaches expert-level annotations, but also is fast during prediction and can run on minimal hardware resources. The HPA dataset, in addition to being extremely unbalanced, is composed of 28 patterns that can appear in mixed forms, resulting in over 500 unique label combinations. For each sample there are four images, the antibody-stained protein of interest (green channel) and three reference channels to outline the cell: microtubules (red channel), nucleus (blue channel) and endoplasmic reticulum (yellow channel). The dataset comprises 31072 samples as public-training and 11702 as private-test.



To address these challenges, we propose a transfer learning approach using well-known Convolutional Neural Networks (CNNs) pre-trained on the ImageNet dataset. There are two primary ways to apply transfer learning from pre-trained CNN architectures: fine-tuning and the other one using the network layers as feature extractors coupled with additional classifiers. In fine-tuning, the entire architecture is retrained, or some layers are frozen while the remaining layers are retrained, which can be computationally intensive.
Our approach takes advantage of the second method, where the network layers are used as feature extractors, and the extracted features are used as input for a Support Vector Machine (SVM) with a linear kernel.
As reported in the literature, the main contributions to HPA image classification operate from two perspectives: the image level and the cell level. Then, these approaches are combined in an ensemble mode to obtain the final classification. We have followed this methodology as well. At both the image and cell levels, we propose a pipeline that begins with extracting feature blocks from the layers of 12 well-known CNNs pre-trained. Feature extraction is performed separately on the four available channels (blue, green, red, and yellow), on the composition of the RGB channels (as suggested in some studies), and on the average of all four channels, resulting in six different feature extractions per sample. We investigate which feature blocks offer the best performance, and to select the sub-optimal feature block, we propose a search strategy based on Genetic Algorithms (GA).
Preliminary results indicate that our approach, which includes Explainable AI (XAI) techniques to enhance model transparency, can achieve good performance with limited computational effort.

SPOKE 3, TASK 1.1

# Phantom studies to develop an AI (artificial intelligence) powered Ultra Low Dose acquisition protocol with optimal lung nodule detection in chest CT imaging

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Dose reduction in CT is a current challenge which can be made possible also using Artificial Intelligence methods. For example, lung cancer screening in at-risk population has been proven to be useful in reducing mortality, but with many problems, among them the exposure of potentially healthy population to ionizing radiation and the difficulties in detecting small nodules. A reduction of the radiation dose delivered in chest CT preserving the image information content is necessary. Recently, the increase in available computing power has allowed the use of iterative reconstruction (IR) methods, which enable better noise management, and the development of CAD (Computer-Assisted Detection) systems. Nowadays, radiomics, consisting in the mathematical extraction of features from biomedical images, represents a further quantitative approach. The synergy between these two techniques could lead to the creation of new hybrid analysis systems capable of tackling the nodule classification task in a reliable way.
The problems related to radiation dose reduction in chest CT and the robustness of radiomic features have been addressed using clinical CT machines and two phantoms: the commercial Catphan phantom, employed in clinical practice for quality control, and the custom Radiomik phantom, specifically designed for radiomics research and not yet characterized. The Catphan CT images have been acquired by means of two clinical CT scanners with several acquisition and reconstruction settings, different computed tomography dose index (CTDI) values and IR blending levels. Image quality was assessed using the Catphan inserts with also a new task-based metric, the detectability index (d'). From the trends of the detectability index versus the acquisition protocols, many "equivalent" protocols capable of providing similar image quality with different CTDI values and IR blending levels have been identified. The repeatability and robustness of the radiomic features extracted from the Catphan inserts were evaluated separately for the images acquired with the "equivalent" protocols and for the other images. The improvement of the robustness of the features extracted from "equivalent" protocols suggests that an upstream harmonization method based on image quality matching may improve the stability of radiomic features. This behavior was verified with the RadiomiK phantom, containing various inserts with different shapes, materials, textures and filling percentages.
The results obtained, especially for the Catphan insert made of polystyrene, whose contrast is similar to that of nodules, indicate that, using high IR blending levels, it could be possible to design ultra low dose acquisition protocols with a dose savings of up to 40% (in comparison with standard protocols) without degrading the information useful for the diagnosis. From the radiomics study conducted with the Catphan it emerged that 78% of the features extracted from the polystyrene insert presents a better robustness category when "equivalent" protocols are considered. From the analysis conducted with the Radiomik phantom it was demonstrated that the image quality based harmonization strategy could improve the robustness of radiomics features, especially for inserts with higher filling percentage, and that following a standardized process for both image acquisition and feature extraction is fundamental to enhance the validity of radiomic model.

# Combining swarm learning and generative adversarial networks for decentralized and privacy-preserving generation of medical images

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Generative Adversarial Networks (GANs) have risen during the past decade as one of Deep Learning (DL)'s most used application models for synthetic image generation in computer vision, and they are nowadays able to generate astonishingly photorealistic images. Several motivations lay at the core of their implementation in precision medicine, such as enlarging already-existing datasets with synthetic samples and, further on, creating fully synthetic datasets that would make it possible to preserve patients' privacy by not sharing any of their data. Nevertheless, reproducing the state-of-the-art performances of GANs in medical imaging is not trivial. Indeed, a major technical challenge when working with medical data is linked to its availability and privacy – which are often related. Restrictions to ensure patients' data privacy are an obstacle to conceiving large and centralized datasets required to train effective GANs, as they need to see a high number and variety of samples in input to avoid overfitting and to reproduce data variability. Furthermore, scientific gaps due to the inherent characteristics of medical images raise other challenges when generating synthetic data. The complexity of their information, lying in their morphometric properties or intensity levels, needs to be preserved in the input of GANs and reproduced in generated images. In this context, the medically realistic nature of synthetic samples needs to be properly evaluated, i.e., not only using classical metrics for generative models – Fréchet Inception Distance (FID) and inception Score (IS), but showing them to a medical doctor for expert evaluation. To answer challenges linked to data privacy and availability for synthetic data generation, we propose a framework combining Swarm Learning (SL), a decentralized, privacy-preserving machine learning framework developed by HPE, with GANs. SL trains a local model on each node (data center) of the swarm network and updates a decentralized model by merging local models' parameters at every given synchronization interval. Only the local parameters are shared during the merging step, so data samples remain visible only locally on their node. Applied to GANs, it enables learning from different data centers while maintaining privacy, increasing the number of data samples and their variability to generate higher-level synthetic images. To validate this framework, we implemented a simple Conditional-GAN (C-GAN), as a toy example, using the MNIST dataset (handwritten 28x28 digits) under a very simple swarm network configuration of 2 nodes set within the same host server. Using different data splits on the nodes, we found out, as expected, that the decentralized model generates synthetic samples with greater variability and tends to be less overfitting than the local models. This initial validation confirmed our intuitions and paves the ground for further experiments, using a more complex model jointly with higher-resolution medical data. Specifically, we are interested in leveraging BigGAN with 2D 256x256 brain MRI data from the ADNI dataset. After Investigating and implementing a more accurate metric system, medically speaking, we expect to observe higher diversity and robustness among synthetic samples generated by the decentralized models.

# SESSION 2.2

# Development and evaluation of bispecific T-Cell-redirecting engagers for enhanced anti-tumour and antimicrobial activity

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T cells bearing the γσ T cell receptor (TCR), in particular Vγ9Vσ2 T cells, play a pivotal role in the anti-tumour immune response due to their involvement in natural immune surveillance and pro-inflammatory activity. These γσ T cells recognise phosphoantigens (PAgs) without the need for antigen processing and presentation, and MHC restriction. PAgs are pyrophosphates derived from the microbial non-mevalonate isoprenoid biosynthetic pathway, whereas structurally related pyrophosphates are produced by the mevalonate pathway in eukaryotic cells. Vγ9Vσ2 T cells are found in many haematological and solid tumours, and their presence is often correlated with improved clinical outcomes. Activation of these T cells by exogenous PAgs or upregulation of endogenous PAgs due to infection or tumour transformation triggers their reactivity, albeit with variable sensitivities. γσ T cells exhibit potent cytotoxic and anti-tumour properties both in vitro and in xenograft models in vivo. Upon activation, Vγ9Vσ2T cells secrete interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α), which have direct cytotoxic effects on tumour cells and indirectly enhance anti-tumour responses by stimulating macrophages and dendritic cells. The robust anti-tumour activity and broad reactivity of Vγ9Vσ2T cells against multiple tumour types have led to their investigation as potential therapeutic agents. Results from small clinical trials have demonstrated that Vγ9Vσ22 T cell-based immunotherapy can improve overall survival rates with minimal toxicity, suggesting its potential as an adjunct to conventional cancer treatments.

Bispecific antibodies (bsAbs), which have dual specificity for two different antigens, have been studied primarily in the context of cancer and inflammatory diseases. Bispecific T-cell redirecting engagers (BsTCEs) are a type of bispecific antibody (bsAb) that bind CD3 on T cells and a specific cancer cell antigen, thereby recruiting T cells to target and kill cancer cells. BsTCEs facilitate the formation of an immune synapse between T cells and tumour cells, leading to T cell receptor (TCR) activation, the release of cytotoxic molecules such as granzymes and perforin, and subsequent tumour cell lysis. This method activates T cells via CD3ξ binding in the TCR complex, bypassing the need for major histocompatibility complex (MHC) restriction and TCR epitope specificity.

The aim of this study is to develop and evaluate several bispecific molecules to identify the most effective candidates. After purification and quality control, these bispecific constructs will be tested in vitro for their ability to mediate cytotoxic and microbicidal activities against human macrophages infected with Mycobacterium tuberculosis and to redirect CD3 T cell cytotoxicity towards various tumour cell types. This research aims to enhance the therapeutic potential of BsTCEs in the treatment of cancer and infectious diseases and provide a basis for further clinical development.

SPOKE 5, TASK 2.4

# H-Ferritin and monoclonal antibodies nanoconjugates: anticancer activity and biodistribution investigations

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During the last decades, immunotherapy has significantly developed as an alternative modality to treat cancer malignancies. In contrast to the other therapeutic concepts, immunotherapy aims to eliminate the primary mass of cancer and relevant peripheral metastases by re-educating the host immune system to recognize the cancer cells as "non-self" and consequently react against them. Many different strategies have been reported in the literature and, among these, antibody-based therapy is one of the most successful for the treatment of highly aggressive cancer subsets. Indeed, monoclonal antibodies (mAbs) can promote tumor killing both by immune cells activation through a mechanism known as antibody-dependent cellular cytotoxicity (ADCC) and by the direct inhibition of tumor proliferation and survival pathways. However, the therapeutic efficiency of mAbs is still limited by problems related to their poor pharmacokinetics and crossing of biological barriers. Nanoparticles proved to be a promising strategy to overcome mAbs' limitations because they can be both functionalized on the surface with specific ligands and exploited as vehicles for drug delivery. Among them, H-ferritin (HFn), a recombinant form of human apoferritin, has been extensively studied because it is biocompatible, can be loaded with drugs and exhibits tumor targeting by recognition of transferrin receptor 1 (TfR1), overexpressed in 98% of human solid cancers. After demonstrating that HFn is an efficient carrier to enhance the blood-brain barrier (BBB) crossing of mAbs without the loss of their antitumoral activity on 2D tumor models, we focused on the evaluation of the activity of HFn-mAb nanoconjugates on 3D cellular models. As a three-dimensional model we chose to use spheroids because they are able to closely mimic the main features of human solid tumors. First experiments were carried out to optimize the formation of cancer spheroids of glioblastoma and breast cancer cell lines, to produce a recombinant form of human apoferritin with a reduced endotoxin content and to optimize the conjugation reaction between HFn and the monoclonal antibody Cetuximab (CTX). Then we investigated the ability of HFn-mAb nanoconjugates to trigger the ADCC monitoring over time the increase in mortality resulting from the activation of the apoptosis mechanism. The data obtained so far with 3D models demonstrated the efficacy of HFn-mAbs to trigger an anticancer effect and activate the immune system. Recently we also produced a labelled version of HFn-CTX in order to study its distribution in 3D cultures and murine models. The biodistribution experiment conducted in vivo revealed that the nanoconjugate signal was clearly visible at the tumor site within 1 hour after injection and remained highly detectable after 3 hours. Besides tumors, the major accumulation of nanoparticles was observed in the liver, in accordance with its physiological detoxification role. In the future, we'll try to co-administer our HFn-mAb with doxorubicin or cisplatin-loaded ferritin to combine the immune system activation with the chemotherapeutic activity of these drugs and also to confirm HFn-CTX in vivo anticancer activity.

SPOKE 6, TASK 4.1

# Digital modelling of the conduction system of the heart: the atrioventricular node

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The atrioventricular node (AVN) plays a crucial role in the heart by controlling the electrical impulse transmission between the atria and ventricles. It also acts as a secondary pacemaker, ensuring a backup system for heart rhythm. Despite its importance, understanding the mechanisms underlying AVN pacemaking remains limited. This research addresses this gap by presenting a novel computational model that captures the pacemaker activity of AVN cells.
Current computational models for AVNs are rare, with only a rabbit model and a limited mouse model lacking calcium handling mechanisms. This study builds upon the existing model by incorporating more realistic cellular compartments and simulating calcium dynamics. We have achieved this by leveraging data from electrophysiological experiments on AVN cells.
Our model integrates detailed electrophysiological data and incorporates key ionic currents, including the funny current, L-type and T-type calcium currents, rapid and slow delayed rectifier potassium currents, and the sodium-calcium exchanger current. One of the significant advancements of the model is the incorporation of a dynamic representation of calcium handling within the cell. This includes the calcium release from the sarcoplasmic reticulum (SR), the uptake by the SR Ca2+-ATPase (SERCA), and the buffering by intracellular proteins. This detailed calcium cycling mechanism is crucial for capturing the interplay between membrane potential and intracellular calcium dynamics, which are vital for the pacemaking function.
The new model successfully replicates key features of the AVN action potential, including its characteristic shape and slow firing rate. This accomplishment signifies the model's ability to accurately represent AVN cells' electrical activity. Furthermore, we have employed the model to simulate the effects of blocking specific ionic currents known to be involved in AVN pacemaking. The model's response aligned with experimental observations, highlighting its potential for investigating the intricate mechanisms governing pacemaker activity.
This novel computational model offers significant advantages for studying the heart's electrical conduction system. It provides a powerful tool for researchers to: i) gain deeper insights into the ionic mechanisms responsible for AVN pacemaking; ii) explore the influence of various factors, such as drugs or genetic mutations, on AVN function; iii) develop and test new therapeutic strategies targeting AVN-related arrhythmias.
This research paves the way for a more comprehensive understanding of cardiac rhythm regulation by offering a platform to analyze and predict the electrical behavior of AVN cells. This knowledge can ultimately pave the way for improved diagnostics and treatment of heart rhythm disorders.

# Biosensing extracellular vesicles and cells in biological fluids: emerging nanotechnologies for liquid biopsy

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In recent years, there have been rapid advancements in the field of liquid biopsy, particularly in utilizing Extracellular Vesicles (EVs) such as exosomes for diagnostic and prognostic purposes. EVs, comprising membranous vesicles of varying sizes and cellular origins, transport macromolecules between cells. Exosomes, particularly, have garnered attention due to their small size (40-100 nm) and their ability to carry genetic material and other molecules. Analysis of exosome cargo has shown promise in precision medicine, offering diagnostic and prognostic insights as well as therapeutic avenues, especially in oncology.

Liquid biopsy, which involves studying circulating markers in peripheral blood for tumor genetic and molecular characteristics, is emerging as a promising approach. While cancer cells and cell-free DNA were initially the focus, exosomes have gained traction due to their role in mediating intercellular communication and their presence in various biological fluids. Isolating and analyzing exosomes allow for early tumor detection and monitoring without invasive procedures.

The project aims to develop biosensors using luminescent nanostructures for optical detection and isolation of exosomes, facilitating liquid biopsy. The project involves chemical and physical researchers in designing nanostructures functionalized with antibodies for specific exosome recognition.

High-crystallinity $Y_2O_3$ nanoparticles (NPs) doped with $Er^{3+}$ ions were functionalized by using a pegylation procedure. The NPs were thoroughly characterized using transmission electron microscopy (TEM), inductively coupled plasma mass spectrometry (ICP-MS), and photoluminescence measurements. The pegylated NPs were studied both from a toxicological perspective and to demonstrate their internalization within HCT-116 cancer cells. Cell viability tests allowed for the identification of the "optimal" concentration, which yields a detectable fluorescence signal without being toxic to the cells. The internalization process was investigated using a combined approach involving confocal microscopy and ICP-MS. The obtained data indicate the efficient internalization of NPs into the cells with emission intensity showing a strong correlation with the concentrations of NPs delivered to the cells. Moreover, our research activity in the biosensing platform field is currently focused on evaluating sensors designed to identify specific microRNAs from U87 exosomes, enabling real-time detection of exosomal miRNAs, eliminating the need for RNA extraction and target amplification, and simplifying direct and quantitative measurement processes. Simultaneously, we are exploring alternative approaches to modify particles with specific antibodies (CD81+) to target a particular subset of exosomes present in the bloodstream. Through this collaborative effort, the project seeks to enhance professional relationships among researchers, fostering a robust network that enriches personal skills and facilitates professional growth, particularly for young researchers. Additionally, achieving project objectives holds the potential for developing low-cost systems for early cancer diagnosis. Leveraging the communication capabilities of exosomes, labeled EVs could also be utilized for targeted drug delivery, optimizing treatment effectiveness, and reducing costs for healthcare services.

SPOKE 6, TASK 1.1

# Detection of extracellular vesicles with electrolyte-gated organic transistor biosensors

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Bio-recognition is a fundamental and specific mechanism in biological processes in living systems, and it is widely exploited in technological and health applications. Organic Electronics (OE) is an emerging technology perfectly suited to connect electrical and biological worlds: the biocompatibility of many materials used for the fabrication of OE devices, the ability to communicate with living systems through both ionic and electronic currents, and the high sensitivity to small variations of potential differences make OE a perfect platform for the realization of specific biosensors. Extracellular vesicles (EV) are emerging as potential biomarkers for early diagnostics of a number of cancer types. EV are heterogeneous populations of membranous vesicles of different diameters (tens to hundreds of nanometers) and cellular origin which can be detected in several bodily fluids and can provide minimally invasive information about health status. We characterized different sensing architecture based on electrolyte-gated organic transistors (EGOTs) in model solutions containing concentrations of lipidic micelles, moving from top-gate configuration to planar gate, in order to maximize the sensitivity. We tested a number of functionalization strategies of the sensing electrodes, and the response of the platform in a microfluidic system.
The preliminary results demonstrate that EGOTs can be used as sensing platforms for the detection of lipidic micelles in model solutions. The following steps will be the characterization of the sensing response in real samples.

# BMP7 promotes cardiomyocyte regeneration

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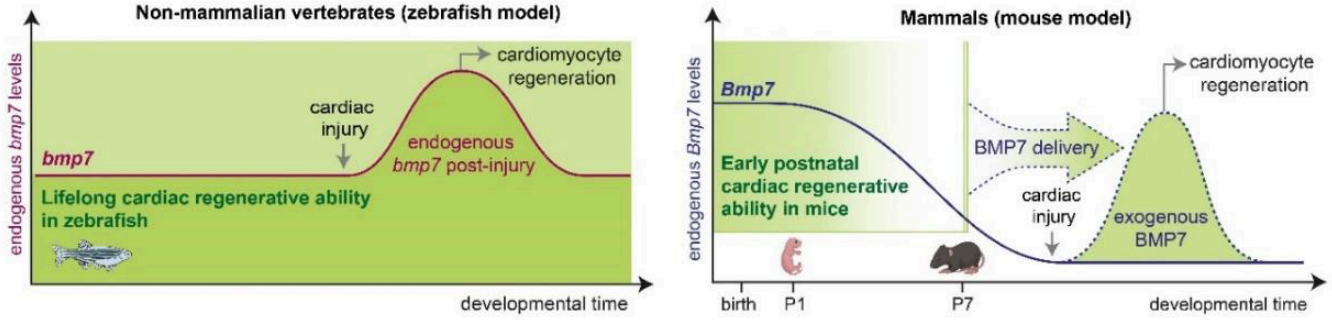
Certain species of the animal kingdom, including zebrafish, exhibit the astonishing capability of efficiently regenerating the injured heart by triggering the proliferation of pre-existing cardiomyocytes. While neonatal mammals share this innate potential, it is suddenly lost during early postnatal development. Consequently, severe heart injury in adult mammals leads to massive loss of cardiomyocytes, which are unable to regenerate, thereby resulting in scarring and impaired cardiac function. This study aims to investigate whether the postnatal regulation of specific growth factors may concur with the loss of cardiomyocyte regenerative potential in mammals. By performing bioinformatical analysis, we unveiled that several previously identified pro-regenerative growth factors, including RANKL, IL6, IGF2 and NRG1, exhibit declining expression levels in early postnatal life in the mouse model, parallelly with the decline in cardiomyocyte regenerative potential. We also identified novel potential pro-regenerative factors declining in expression and driving cardiomyocyte proliferation. Among them, a factor belonging to the bone morphogenetic protein family, named BMP7, triggered the most notable effect. Knockdown or knockout of Bmp7 reduced the proliferation of cardiomyocytes from neonatal mice in vitro and from regenerating zebrafish in vivo, demonstrating the key role of BMP7 in sustaining cardiomyocyte regeneration in the regenerative stages of these models. In contrast, bmp7 overexpression in zebrafish hearts or delivery to juvenile and adult mice, increased cardiomyocyte cycling in vitro and in vivo after cardiac injuries. Finally, we revealed that BMPR1A/ACVR1 and ACVR2A/BMPR2 type I and type II receptors along with canonical SMAD5 and non-canonical ERK and AKT downstream signalling players mediate the cardiomyocyte pro-regenerative effect of BMP7. Overall, we highlight BMP7 delivery as a promising strategy to trigger cardiomyocyte regeneration following injury. We are currently focusing on other members of the BMP family to assess potential synergistic or opposing effects.




SPOKE 5, TASK 4.2

# Quantitative evaluation by digital pathology of immunohistochemical expression of CK7, CK19, and EpCAM in advanced stages of NASH

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Nonalcoholic Steatohepatitis/Nonalcoholic Fatty Liver Disease (NASH/ NAFLD) is the most recurrent chronic liver disease. NASH could present with a cholestatic (C) or hepatic (H) pattern of damage. Importantly, C pattern is associated to a worse prognosis than H pattern. In the present study, we used digital pathology to compare the quantitative results of digital image analysis by QuPath software (Q-results), with the semi- quantitative results of observer assessment (S-results) for cytokeratin 7 and 19, (CK7, CK19) as well as Epithelial Cell Adhesion Molecule (EpCAM) expression. Patients were classified into H or C group on the basis of the ratio between alanine transaminase (ALT) and alkaline phosphatase (ALP) values, using the "R-ratio formula" (R = ALT/ALT at the upper limit of normal)/ (ALP/ALP at the upper limit of normal). Q- and S-results showed a significant correlation for all markers ($p < 0.05$). Q-EpCAM expression was significantly higher in the C group than in the H group ($p < 0.05$). Importantly ALP, an indicator of hepatobiliary disorder, was the only biochemical parameter significantly correlated with Q-EpCAM. Instead, Q-CK7, but not Q-CK19, correlated only with γ Glutamyl-Transferase(γGT). Of note, Stage 4 fibrosis correlated with Q-EpCAM, Q-CK19, and ALP but not with γGT or ALT. Image analysis confirms the relation between cholestatic-like pattern, associated with a worse prognosis, with increased ALP values, EpCAM positive biliary metaplasia, and advanced fibrosis. We observed that increased EpCAM expression was the main immunohistochemical feature to distinguish C from H pattern in NASH. These preliminary data could be useful for the implementation of AI algorithms for the assessment of cholestatic NASH.

# Digital detection of weak and/or focal p16 immunohistochemical expression in cervical biopsies and correlation with high-risk HPV

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In cervical biopsies, only diffuse and strong immunohistochemical staining for p16, pattern named "block-like", has a diagnostic value for high risk Human Papilloma Virus (HPV) infection. "Weak and/or focal" (w/f) p16 expression is commonly considered nonspecific. The aim of the present study was to investigate the presence of high risk HPV (hrHPV) DNA by LiPa method and by CISH in areas showing w/f p16 expression. We found the presence of hrHPV16, 18, 31, 33, 51 by CISH in a group of 20 cervical biopsies showing w/f p16 expression, some with increased Ki67, and in 10 cases of block-like expression, employed as control. Digital pathology, by using QuPath software package, was employed to establish the minimum threshold value of p16 staining, at which the w/f positivity was considered relevant. hrHPV-CISH nuclear positivity was encountered in 12/20 cases of w/f p16 expression (60%). Different patterns of nuclear positivity were identified, classified as punctate, diffuse and mixed, with different epithelial distributions. Our results, albeit in a limited casuistry, confirm the presence of hrHPV and show the presence of HPV in an integrated status, highlighted by CISH, in cases of w/f p16. This could suggest the necessity of a careful follow-up of the patients with "weak" and/or "focal" immunohistochemical patterns of p16, mainly in cases of increased Ki67 cell proliferation index, supplemented with molecular biology examinations. Our work represents a clue for employing digital pathology in diagnostic of cervical biopsies, highlighting the w/f p16 areas.

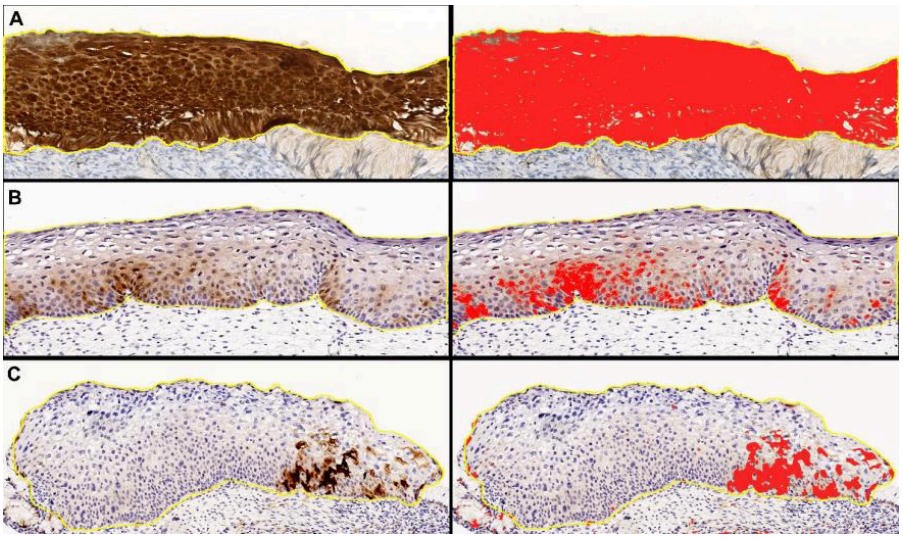


Figure 1. Representative images of "digital pathology evaluation" performed with QuPath software on p16 digital slides, with original DAB-stained images (left) and the relative digital detection of DAB signal in red (right). (A) Block-like positive case. (B,C) Weak and/or focal positive cases.

# Deep profiling of Clostridia in the intestinal community: phylogenomics and identification of butyrate producing species

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In order to investigate gut species belonging to the class Clostridia, a set of 51 publicly available metagenomes of fecal samples from healthy subjects from 4 different countries was collected from NCBI Sequence Read Archive (SRA). The microbial composition of the metagenomes was assessed using the taxonomic sequence classifier Kraken2 (Wood et al. 2019), followed by Bracken analysis. To obtain a higher resolution of the microbial composition, we utilized the Kraken2-custom database provided by the Unified Human Gastrointestinal Genome (UHGG) version 2.0.1 consisting of 4744 species representatives retrieved from the human gut microbiome.
Clostridia encompassed 1899 features with complete taxonomic lineages, among which 404 were ascribed to species with recognized binomial nomenclature. In terms of relative abundance within the class Clostridia, the recognized species ranged from 18 to 75%.
The majority of Clostridia species belonged to the orders Oscillospirales (755) and Lachnospirales (512), that covered the highest relative abundance (19.6 and 18.4% of bacteria). The others were included in Christensenellales (248), Peptostreptococcales (76), Clostridiales (47), Monoglobales (13), and Eubacteriales (6).
Phylogenetic reconstruction of the 411 recognized species of Clostridia, performed through identification, concatenation, and alignment of the 120 GTDB ubiquitous single-copy proteins, revealed that a few lineages speciated in hundreds of different species. The rooted phylogenetic tree indicated that, with the exception of Eubacteriales, the other 10 orders of intestinal Clostridia originated from the same lineage, that diverged into two major branches A and B. In the lineage A, a solid phylogenetic split generated Peptostreptococcales and Tissierellales, collectively encompassing 76 recognized species. The lineage B, more extensive and diverse (331 species), included the orders Clostridiales, Lachnospirales, Christensellales, Oscillospirales, and 4 other putative orders containing a few genera and species.
The capability of these species to produce butyrate and to positively contribute to the health status of the host was assessed according to the presence of the genes buk and but in the reference genomes of the recognized Clostridia species, encoding key enzymes in the catabolic pathway leading to the butyrate production. All the species of the orders Eubacteriales, Peptostreptococcales, and Clostridiales, and most of the species encompassed in the orders Tissierellales were expected to produce butyrate. On the other hands, butyrate production was quite rare in Christenlalles (with the exception of species of the genus Christensenella and Avichristensenella) and not predicted in Monoglobales. Iin the main orders Lachnospirales and Oscillospirales, the ability to produce butyrate seemed associated to some evolutive branches.

# Evaluating the impact of W8 ARRAY technology on pharmaceutical efficacy assessment

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The development of effective pharmaceutical treatments is challenged by complex and costly research processes, as well as the limitations of traditional drug testing models, such as animal studies. These challenges are being addressed by emerging technologies that promise to enhance the accuracy and efficiency of drug efficacy assessments. The W8 ARRAY technology represents one such advancement. This platform integrates label-free organoid structural quantification with epi-fluorescent investigation, alongside automated liquid handling and protocol automation.
W8 ARRAY uniquely enables the multiparametric quantification of organoids, taking into account various structural variables like size, compaction, and weight. Traditional approaches typically focus on producing uniform-sized samples, which may not fully capture the complexities of organoid behavior. By including a broader range of structural variables, W8 ARRAY offers a more detailed analysis of organoid physiology, which is critical for accurately assessing the permeation and cytotoxicity of pharmaceutical compounds.
The application of W8 ARRAY in drug development processes facilitates a more precise normalization of drug effectiveness results against these structural variables. This methodological advancement can potentially streamline the drug discovery process by providing researchers with deeper insights into the structural and functional characteristics of organoids. It also aims to reduce the economic burden associated with developing and testing drugs that fail to meet efficacy standards.
Furthermore, W8 ARRAY contributes to the advancement of personalized medicine by supporting the development of treatments tailored to individual patient profiles. This is achieved through detailed analyses of organoid structures, which can inform more customized therapeutic strategies.
This presentation will explore the operational capabilities of W8 ARRAY and discuss its potential to impact the field of pharmaceutical research. The focus will be on how this technology can help overcome some of the inherent challenges in drug development, thereby supporting the broader goals of precision medicine.

# Glucose-functionalized hybrid gold/carbon nanodots exploiting the Warburg effect for multimodal theranostic treatments of breast cancer

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The non-specific distribution and mechanism of action of current diagnostic and therapeutic anticancer approaches limit their efficacy, causing severe side effects that negatively affect patients' quality of life and prognosis. Nanotechnology can address the urgent need for targeted antitumoral strategies by providing nano-scaled tools engineered to target the tumor site and exhibit several functionalities. Structures presenting an ultrasmall size (<10 nm) are desirable to ensure the efficient diffusion of the therapeutic tools through the stiff tumor microenvironment (TME), besides their preferential accumulation driven by the EPR effect. Tumor targeting can be further achieved by modifying the surface of the nanoparticles with moieties that selectively bind cancer cells by recognizing their distinctive biochemical characteristics. For instance, cancer cells exhibit abnormal metabolic pathways, including alterations in glucose metabolism that lead to a massive glucose demand. This phenomenon, known as the Warburg effect, offers an appealing opportunity for innovative strategies that use glucose as a targeting moiety. Nanosystems should also be endowed with bioimaging contrast properties to enable cancer diagnosis, monitoring, and therapy simultaneously. This multifunctional approach, denoted as cancer theranostics, can be achieved using hybrid gold/carbon nanomaterials, which leverage the optical and size-related properties of carbon nanodots (CDs) with the X-ray attenuation properties of gold to implement a multi-modal imaging approach that benefits from the synergic combination of fluorescence imaging (FLI) and computed tomography (CT). Herein, we designed a unique hybrid gold/carbon structure obtained by introducing gold seeds among the molecular precursors of carbon nanodots during their solvothermal synthesis. This original synthetic strategy led to unique AuCDs of 6 nm diameter incorporating bio-eliminable gold seeds, which can be easily excreted by renal clearance after the degradation of the carbon structure, thus avoiding the typical accumulation phenomena responsible for the toxicity of gold nanoparticles. Subsequently, we employed an azido-alkyne click Huisgen cycloaddition to functionalize the nanoparticle's surface with 2-deoxy-D-glucose as a targeting moiety, exploiting the Warburg effect for achieving the active targeting towards cancer cells. The resulting conjugate, named AuCDs-PEG-Glu, presents both remarkable X-ray attenuation capability and tunable fluorescence emission in the blue-green region, allowing for FLI/CT bi-modal imaging of cells, potentially valuable in image-guided cancer theranostics. The self-fluorescence of the obtained AuCDs-PEG-Glu was exploited to prove the glucose-dependent entry of the nanosystem into cells by monitoring the cellular internalization through FLI, enlightening a preferential uptake in breast cancer cells due to the Warburg effect. The demonstrated cancer-targeting proficiency together with the self-tracking ability by multi-modal imaging clearly showcases the high potential of the proposed nanosystem for a broad range of applications in precision anticancer theranostics, embracing both diagnostic and therapeutic purposes.

SPOKE 6, TASK 4.2

# Exploiting circulating tumor DNA (ctDNA) to intensify the adjuvant (adj) or post-adj treatment of stage III and high-risk stage II resected colon cancer patients (pts): the ERASE-CRC project by GONO.

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Three or 6 months of fluoropyrimidines plus oxaliplatin is the standard adj therapy for stage III and high-risk stage II colon cancer, but 20-25% of pts relapse. CtDNA is a promising biomarker of minimal residual disease (MRD) after primary tumor resection and a potential guide in the adj treatment decision-making process. Indeed, ctDNA-positivity after surgery or at the end of adj therapy is associated with higher risk of recurrence. Intensifying the adj and/or the post-adj treatment could be a valuable strategy in these cases. CtDNA clearance has been suggested as a predictor of long-term clinical outcome.
ERASE-CRC (NCT05062889) is a prospective, open-label, multicentre study, including 3 phase 2 trials enrolling ctDNA-positive (+) pts with radically resected stage III or high-risk stage II adenocarcinoma of the colon or intraperitoneal rectum. CtDNA is centrally assessed through a tissue-informed method, F1Tracker. In the ERASE-CRC Part 1 study, pts that are ctDNA+ after surgery (2-6 weeks) are randomized 1:1 to 6 months of either FOLFOX or CAPOX at investigators' choice (arm A) versus FOLFOXIRI (arm B). In the ERASE-HER2 study, ctDNA+ pts with HER2 amplified and RAS wild-type tumors received FOLFOX plus trastuzumab and tucatinib for 12 cycles. In ERASE-CRC Part 2 study, ctDNA+ pts after the end of an oxaliplatin-based adj therapy (either in the context or outside Part 1 or ERASE-HER2) are randomized 1:2 to observation (arm A) or trifluridine/tipiracil for 6 cycles (arm B). The primary endpoint is the ctDNA clearance rate (CR) after the adj (Part 1 and ERASE-HER2) or the post-adj treatment (Part 2). 300 pts will be randomized in Part 1 to detect a 15% difference in ctDNA CR between arms B and A (65% vs 50%), and 1-sided alpha and beta errors of 0.05 and 0.2, respectively. 18 pts will be enrolled in ERASE-HER2 to observe a ctDNA CR of 80% with the targeted treatment and ctDNA clearance should be observed in 13 cases to consider the strategy promising. Finally, 159 pts will be randomized in Part 2 to detect a 15% difference in ctDNA CR between arms B and A ( 20% vs 5%), and 1-sided alpha and beta errors of 0.05 and 0.2, respectively.
About 42 Italian Oncology Units are involved. From March 2023 to April 2024, 282 pts were screened into ERASE Part 1, with a success rate of 95%. 16 of the 51 patients (31%) with positive liquid biopsy had metastatic disease revealed by post-operative CT scans. Instead, 147 patients were screened for the study's Part 2, which had a lower failure (1%) and positivity (10%) rate than Part 1. In this case, radiologically evident metastatic disease was present in only 13% of positive patients. Currently, 30 and 11 patients have been randomized in ERASE-CRC Part 1 or 2, respectively.

# 4D Flow MR Imaging for blood velocity field validation: a preliminary study in atrial fibrillation patients

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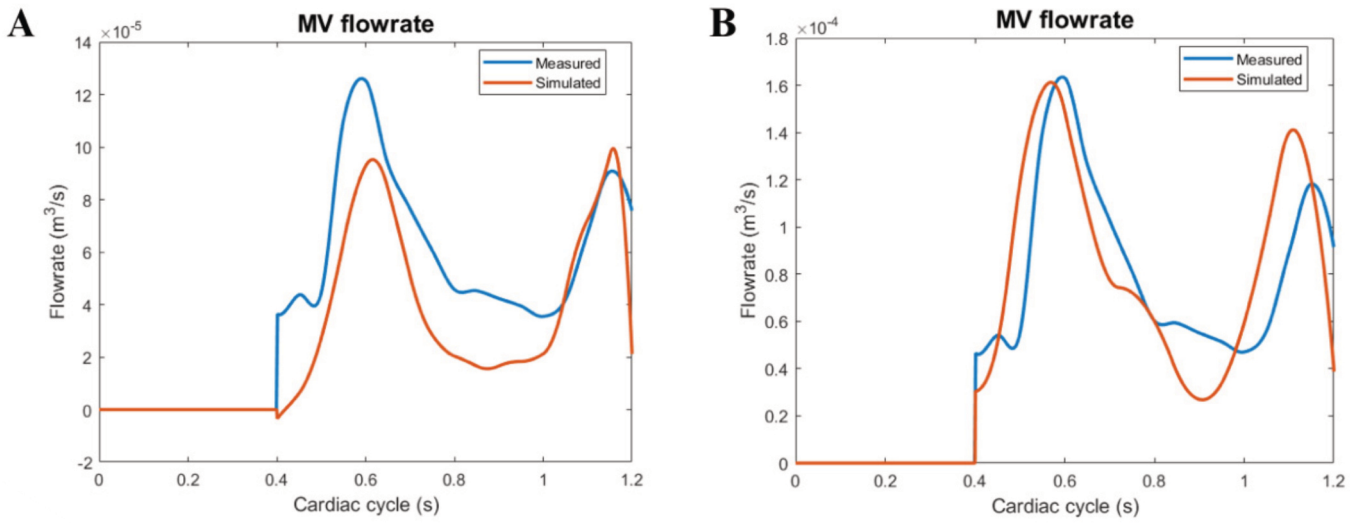
Computational fluid dynamics (CFD) represents a unique tool to simulate fluid dynamic conditions on a complex system, such as the cardiac blood flow in the heart chamber, in a noninvasive, fully controllable, and reproducible way. Unfortunately, validation of the simulation results is still an open issue.
The purpose of this preliminary study was to obtain an initial validation of a patient-specific computational model comparing the simulated blood velocity field in the left atrium (LA) from CFD with the velocity field provided by 4D flow MRI data. To achieve this goal, we selected the simulation context best suited to the specific clinical question.
Analysis was performed in three atrial fibrillation (AF) subjects. 4D flow MRI data and dynamic CT data were processed to derive the LA anatomical and displacement models from which two distinct dynamic models were computed, representing the computational domains for the CFD simulations.
Patient-specific boundary conditions, in terms of pulmonary veins flowrate, were extracted from 4D flow MRI velocity field and applied to the CFD simulations. For each AF patient, we ran two simulations considering different computational domain: the 4D flow MRI derived model and the CT derived model.
For the purpose of the CFD validation, the focus was to verify how accurately the flowrate at the mitral valve reproduced the one measured from the 4D flow MRI, considered as gold standard.
In all the study subjects, the simulations revealed two recurrent patterns. First, the flowrate simulated with the model derived from 4D flow MRI (Figure 1(A)) turned out to have a better time synchronization with the measured flowrate compared to the simulation results obtained with CT model (Figure 1(B)). Second, similarities in the amplitude of measured and simulated flowrates seemed to be better represented when CT models were considered compared to 4D flow MRI.
This preliminary study suggests that computational domains affect CFD simulation results, and timings and amplitudes of the simulated blood velocity fields should be evaluated also considering the different anatomical models. Being available 4D flow MRI as reference blood flow, a large scale testing and validation of CFD models is required to obtain a correct interpretation of simulation results.




Comparison between measured and simulated MV flowrate from 4D flow MRI anatomical model (A) and CT anatomical model (B) in one AF patient.

SPOKE 4, TASK 2.3

# NV914 molecule as translational readthrough inducing drug: a study on cystic fibrosis G542X stop murine model

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Cystic fibrosis (CF) is an autosomal genetic disease caused by mutations in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene. Among the about 2500 mutations associated with CF, nonsense or stop mutations (class I CFTR mutations) give rise to a premature termination codon (PTC) in the mRNA sequence, leading to premature translation termination and the consequent absence of a functional CFTR protein, resulting in more severe symptoms. These mutations affect 10% of CF patients worldwide and 20.5% of the patients in Italy (Reg. ita. FC 2019-2020). Nowadays there is no cure for this genetic defect, the suggested therapeutic approach involves the rescue of protein synthesis. Developing new drugs focused to CFTR gene mutation classes is an important goal of precision medicine. Translational Readthrough-Inducing Drugs (TRIDs) are a novel class of compounds that promote readthrough of the PTCs, allowing the synthesis of a full-length functional protein.
Recently, three new patented TRIDs (NV848, NV914, NV930) have been proposed by our research group, and validated by several in vitro assays, for the rescue of the expression of the CFTR protein. Acute toxicity studies in vivo demonstrated that the same molecules are safe and well tolerated in mouse models.
In this study, we evaluated the translational readthrough potential of the NV914 molecule in vivo using the CFTRG542X/G542X murine model, which carries the G542X stop mutation.
Five CF mice were chronically administered by oral gavage for 14 days with 60 mg/kg of NV914 or of PTC124 (Ataluren) as a readthrough reference compound. Body weight was monitored during the treatment period, and at the end of the treatment, the animals were sacrificed, and their organs were harvested.
We assessed the rescue of CFTR expression in mouse lung tissue. RT-qPCR showed a partial stabilization of CFTR mRNA transcript in NV914- and PTC124-treated CFTRG542X/G542X mice compared to untreated ones. Western blot analysis on lung protein extracts showed increased CFTR protein expression in NV914- and PTC124-treated CFTRG542X/G542X mice compared to untreated ones. These results show the efficacy of chronic treatment with NV914 molecule in inducing the translational readthrough of PTCs in the CFTR mRNA and the consequent rescue of protein synthesis.
Our study provides promising results for personalized CF therapy and suggests the potential validation of the NV914 molecule as a TRID for other disorders characterized by the same genetic defect.

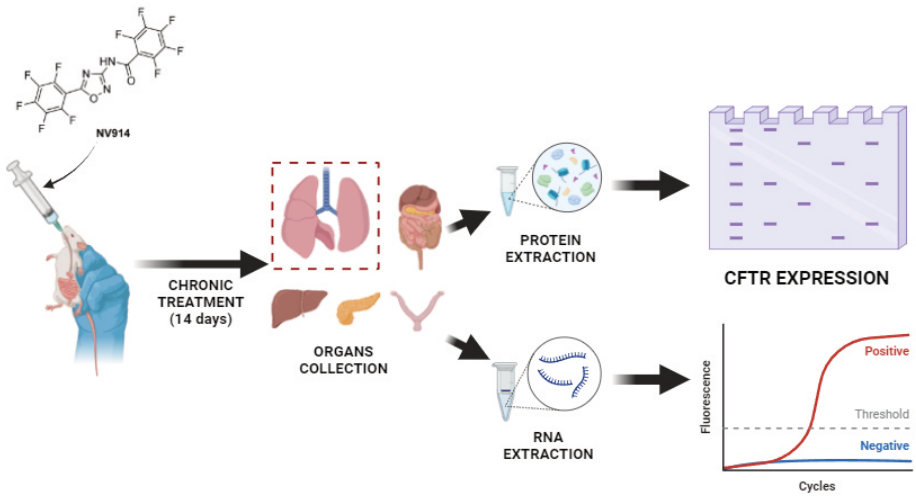


Fig. In vivo evaluation of translational readthrough activity of the NV914 molecule on CFTRG542X/G542X CF murine model.

SPOKE 5, TASK 1.1

# A novel tool for personalized medicine: an AI-based approach for predicting the risk of disease in fascioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) (MIM#158900) is a genetic muscle disorder caused by a contraction of the D4Z4 repeat region on the 4q subtelomere. It is characterized by progressive weakening and wasting of specific muscles. As a hereditary disease, an accurate risk assessment is crucial for improving genetic counseling, especially in the context of pregnancy planning. This study leverages data from the Italian National Registry for FSHD to develop and validate a Bayesian network model aimed at enhancing disease risk prediction.
The Italian Clinical Network for FSHD established the Italian National Registry for FSHD collecting data from over 3500 patients and their relatives, including molecular and clinical evaluations. Since 2009, with the introduction of the Comprehensive Clinical Evaluation form, molecular analysis has been accompanied by clinical evaluation based on standardized evaluation protocols. The Mediator Environment for Multiple Information Sources (MOMIS), which is the web platform developed for the registry data management, integrates this data from 14 neuromuscular clinics across Italy. The MOMIS framework played a crucial role in FSHD research, by providing high-quality data collection and facilitating genotype-phenotype studies.
Overall, the Italian National Registry for FSHD provides a comprehensive dataset, including both genetic and clinical data, as well as family history. Moreover, the availability of family trees for each patient allows us to integrate the family study with the degree of kinship of each family member.
The primary aim of our study is to develop a statistical model to be applied to FSHD patients who are planning a pregnancy, to predict the risk of developing FSHD and its expected severity in their potential newborns. A Bayesian network approach is designed. Bayesian networks are Artificial Intelligence (AI) models that represent a set of variables and their conditional dependencies via a directed acyclic graph. This approach is particularly suited for complex diseases like FSHD, where multiple genetic and environmental factors interact. The Bayesian network will be structured by using a combination of expert knowledge and data-driven approaches. The nodes and edges will be based on established medical and genetic insights into FSHD. The model will incorporate key variables such as age of onset, disease duration, severity of the symptoms, D4Z4 allele dimension, and family history of FSHD. The probabilities of developing the disease given the observed data on the parents and their relatives will be computed applying Bayesian inference. Remarkably, the probabilistic nature of the model allows for updating risk predictions as new data become available, making it a dynamic tool for ongoing risk assessment.
At its current state, the model has been trained on a reduced subset of patient and its performance is under evaluation. Future work will focus on refining the model with larger training dataset and exploring the application of more sophisticated structures and features. By doing this, we expect to improve the model performance, in order to obtain a reliable tool to guide decision-making and genetic counseling.

SPOKE 2, TASK 1.3

# Clustering microbiome data via mixtures of generalized linear latent variable models

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Microbiota plays a crucial role in human health, and it is influenced by variations in environmental or health conditions. Next Generation Sequencing technologies have allowed the exploration of the microbiome without isolation and culturing. However, the analysis and translation of microbiome data into meaningful biological insights is challenging because of the nature of the data, that are compositional, high dimensional, sparse, and over-dispersed. The gut microbiome can vary from individual to individual, and microbiome communities can be grouped to point out community types linked to different ecosystems or health states. In the literature many clustering techniques able to take the microbiome features into account have been developed. Among these techniques there are model-based clustering methods (e.g. mixtures of Von Mises-Fisher distributions, Dirichlet Multinomial mixtures, Latent Dirichlet Allocation, cosine distance-based mixtures) and partitioning and hierarchical methods, such as spherical K-means, Partitioning Around Medoids (PAM) and Ward's method. However, most of these methods do not (explicitly) model the dependence between taxa. This is the reason why we propose to use mixtures of generalized linear latent variable models for multivariate count data. Specifically, in order to identify clusters of individuals who share the same gut microbiome composition and to simultaneously account for genera correlation, we propose a novel mixture model that describes the taxa counts as Poisson random variables whose parameters are generated by a common factor latent variable model. The proposal accuracy has been tested on simulated data before being applied to microbiome data. In particular, the model capability of identifying the true number of clusters (k) and latent factors (q) is evaluated for different settings; in addition, the ability of recovering the true clustering structure (with known k and q) is measured via both the median misclassification error (MISC) and the median Adjusted Rand Index (ARI) of several simulation scenarios over many replicates. A performance comparison among the different clustering methods is also displayed. Finally, a real microbiome data analysis on healthy adults coming mainly from US, UK and Australia is considered.

# Digital twins for preventive health in oncological and ageing populations

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According to the World Health Organization, by 2030, the number of people older than 60 will increase by 34% compared to 2020. Due to risk factors such as increased propensity to fall and deteriorated mechanical properties of the bone tissue, the elderly present a markedly increased bone fracture incidence. A fracture represents a devastating event, affecting the quality of life, increasing morbidity and mortality, with impact on the healthcare system. The available clinical gold standards for bone fracture prediction (e.g., T-score) have shown limited accuracy in stratifying subjects at high risk of experiencing a fracture from subjects who are not. In this context, the use of digital twins can be of help, as these technologies enable to test different clinical scenarios in silico, and beforehand.
We hereby present two examples of use of in silico methods to support clinical decision making and management for oncological and elderly patients, respectively.
The first use case is the Bologna Biomechanical Computer Tomography (BBCT-BC), a digital twin technology designed to predict the risk of femoral fracture secondary to a side fall in connection to osteoporosis and tested so far on a cohort of post-menopausal osteoporotic women. The research question herein is whether BBCT-BC can be used to predict femoral fracture in women under treatment for breast cancer, who may have weaker bones due to the drugs they take. The BBCT-BC digital twin combines an image-based finite-element model of the patient's femur (reconstructed from a quantitative CT scan) which allows to identify the femur failure load (causing its fracture) and an analytical model used to simulate over 1 million side falls (and the resulting impact force) in different conditions according to the subject's height and weight and other stochastic input parameters. The absolute risk of fracture at the time of the visit (ARF0) can thus be estimated as the ratio of the impact forces causing fracture and the total number of simulations. We are currently drafting the documentation for the Ethics approval to conduct a clinical study on 300 women undergoing adjuvant hormonal therapy against breast cancer, of whom 150 are also under treatment for osteoporosis.
The second application falls under the category of musculoskeletal modeling, specifically modeling suboptimal muscle control, i.e. in silico methods to estimate muscle forces and activations and in turn joint contact forces typical of subjects affected by neuromuscular or musculoskeletal diseases who tend to adopt abnormal muscle recruitment strategies to perform the activities of daily living. We are currently testing and further developing a new implementation of a stochastic approach (Myobolica) that allows to explore the entire range of physiologically plausible strategies a person may employ to perform a given task. This approach promises to enable the quantification of motor control variability (and its deviation from what can be considered optimal) in a patient and may be used to design effective rehabilitation programs. The open-access Knee Grand Challenge datasets – which include experimental data from an instrumented knee implant – are being exploited to test the tool and optimize its constitutive parameters.

SPOKE 2, TASK 2.3

# Drug response prediction in precision oncology: a cancer organoids analysis

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Organoids have emerged as a powerful tool in cancer research due to their ability to recapitulate the architecture and function of native tissues. Organoids are aggregates of cells that grow in suspension and can reproduce some important characteristics of the original tumor, including its structure, heterogeneous cell compartment, cell interactions with the extracellular matrix, gene expression and drug sensitivity. This study aims to explore the effects of tumor inhibitor treatments on organoid cultures. Organoids were obtained from patient-derived tumor tissues using established protocols. Tissues were mechanically dissociated, and cells made to grow in a medium containing growth factors. The culture conditions were optimized to promote self-organization of the cells into organoids that mimic the histological and genetic characteristics of the original tumors. The growth and morphology of the organoids were monitored by imaging evaluations. To study the responsiveness of organoids to tumor inhibitors, a panel of 31 compounds targeting major oncogenic pathways was selected. These included kinase inhibitors (e.g. cetuximab), immune checkpoint inhibitors (e.g. atezolizumab), chemoterapeutics (e.g. oxaliplatino) and small molecules known to interfere with cancer cell proliferation and survival. A rigorous protocol for data acquisition was developed to ensure reproducibility and accuracy in measuring treatment outcomes. High throughput imaging techniques, including optical microscopy, were used to capture detailed morphological changes and cell viability over time. The results showed a differential sensitivity of tumor-derived organoids to various inhibitors, reflecting the heterogeneity observed in patient tumors. Kinase inhibitors showed a marked reduction in organoid growth and viability in specific subtypes, while immune checkpoint inhibitors elicited a robust apoptotic response in others. Combination treatments often resulted in enhanced efficacy, suggesting potential therapeutic strategies for overcoming drug resistance. In conclusion, this study underscores the utility of organoid cultures as a preclinical model for evaluating tumor inhibitor efficacy and tailoring personalized treatment strategies. The established data acquisition protocol provides a robust framework for systematic and reproducible analysis, facilitating the translation of these findings into clinical applications. The observed variability in treatment responses among organoids emphasizes the need for personalized approaches in cancer therapy, leveraging the predictive power of organoid models to optimize therapeutic outcomes. Future research will focus on quantitative image analysis for the extraction of features such as organoid size, shape, and density. To this end, the use of Machine Learning/Deep Learning models will serve to predict drug response based on organoid imaging, and develop a predictive system to anticipate the effect of one or a combination of two compounds and suggest a more effective treatment approach. Moreover, we will focus on expanding the organoid biobank and integrating multi-omics data to further elucidate the mechanisms underlying drug sensitivity and resistance.

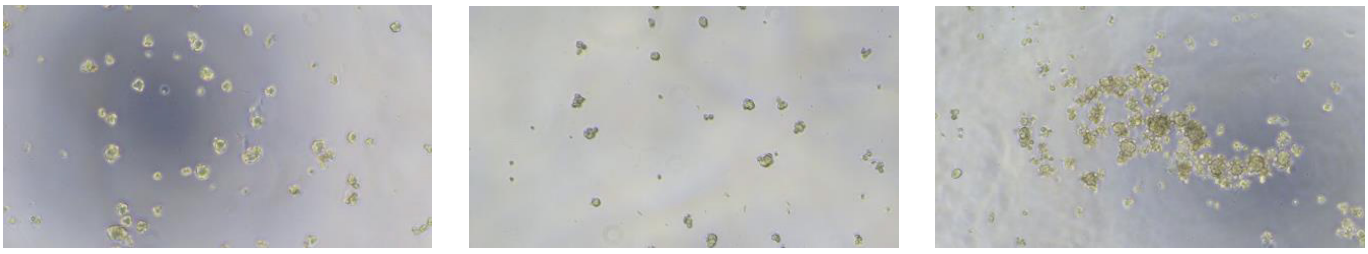

Figure 3: G605 treated with crizotinb at 24, 48 and 72h

SPOKE 3, TASK 4.3

# Atypical nevi in dermoscopy and reflectance confocal microscopy: correlation between immunohistochemistry and diagnostic patterns of atypia.

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Introduction & Objectives: Even if dermoscopy and/or reflectance confocal microscopy (RCM) reveal positive melanoma-associated characteristics, equivocal cutaneous melanocytic lesions can occasionally be diagnosed as "nevi" at histopathology. The use of immunohistochemical (IHC) analysis is used to find markers that distinguish melanoma from naevi. When there is uncertainty about the histological diagnosis, several proliferative markers, including CD271, CD20, CD31, and Cyclin D1, can be examined. These markers have historically been linked to the diagnosis of melanoma.
Materials & Methods: We prospectively detected ambiguous lesions that satisfied at least one melanoma criterion on dermoscopy and/or RCM, with a histological diagnosis of naevus (Prot.# 289\13). IHC was used to evaluate equivocal lesions in order to discover potential biological and morphological markers, such as CD271, CD20, CD31, and Cyclin D1. These markers were then associated with dermoscopy and RCM patterns that supported melanoma. IHC markers were categorized as either nonexistent or in terms of their level of presence (1, 2, or 3 [level 3 exclusive to Cyclin D1]).
Results: Of the 69 patients (69 lesions) that were included, 50.7% of the patients were male and the mean age was 45.7 years (±12.9, range 22.6-71.6). Irregular dots and globules were the most commonly detected feature (72.5%) based on dermoscopic patterns. At RCM, lesions frequently showed pagetoid cells (43.5%; mostly dendritic 25/30), focally distributed (37.7%), in <25% of the RCM image (39.1%), and atypical cells in the dermo-epidermal junction (DEJ; 49.3%). Cyclin D1 (91.3%) and CD31 (95.6%) expression were detected by IHC in the majority of lesions. Infrequently registered were CD20 (23.5%) and CD271 (8.7%). These findings confirm that equivocal naevi also exhibits irregular spots and globules, which are listed in the updated melanoma 7point checklist. Malignant melanoma frequently exhibits pagetoid dendritic cells and atypical cells in the DEJ upon RCM. On sun-damaged skin, dendritic cells have been linked to slow-growing melanoma, or the so-called melanocytic "proliferating attitude". The presence of Cyclin D1, which is thought to promote mitosis and be implicated in the pathophysiology of atypical naevi, verified this proliferating attitude in over 90% of the lesions at IHC. Additionally, nearly all of the atypical naevi in this investigation had the endothelial CD31 marker, which has been linked to enhanced carcinogenesis in melanoma. Cyclin D1 and atypical cells were found to be correlated (p=0.022) by chi-squared analysis, as were atypical cells found in less than 25% of the RCM picture (p=0.029). Atypical naevi included in this study had higher expression of Cyclin D1 than common naevi, which may correlate visually with characteristics tested upon RCM with cytoarchitectural atypia, focally distributed (<25%), which may not translate into a biologically typical type of malignant phenotypes.
Conclusion: The authors suggest that additional information regarding the controversial idea of atypical nevi and their connection to melanoma advancement may be provided by the finding of a small number of localized atypical cells in the DEJ upon RCM and positive IHC markers linked to tumor progression (initialization?).

# Investigating the interactions between genetic factors, lifestyles, and mother-child outcomes to delineate targeted and effective prevention strategies

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The lifespan of humans is influenced by a combination of genetic and non-genetic factors, which may dynamically interact and influence the overall health trajectories. Maternal exposures (i.e., diet, cigarette smoking, physical activity) during the periconceptional period may have a critical impact on adverse outcomes, suggesting the need of novel approaches of precision prevention. In this context, polygenic risk score (PRS) may provide a useful tool to evaluate how the individual genetic variants may affect disease risks. In this scenario, the activities coordinated by Professor Antonella Agodi at the University of Catania aim to perform the whole-genome genotyping analysis in order for the identification of genetic variants that may act as effect modifiers in the relationship between maternal exposome and maternal-child outcomes, with a particular focus on dietary and nutritional factors. To do this, maternal and neonatal biological samples from the "MAMI-MED" cohort, an ongoing prospective study on mother–child dyads from the "Azienda di Rilievo Nazionale e di Alta Specializzazione (ARNAS) Garibaldi Nesima" (Catania, Italy), coordinated by the Professor Antonella Agodi, will be used. At recruitment, maternal lifestyles are collected through structured questionnaires, and the Food Frequency Questionnaire is used to assess maternal diet. The study involves a follow-up at 12, 24, and 48 months to gather information regarding the health, socio-economic status, and lifestyles of the parents, as well as data concerning health, habits, and diet of the child. To date, the MAMI-MED cohort includes a total of 1917 women (median age 31 years). Preliminarily activities, including maternal and neonatal DNA extraction using peripheral blood samples and umbilical cord samples have been conducted for future genotyping analyses. In addition, we conducted a systematic review providing an overview of epidemiological studies exploring the interactions between genetic variants, maternal dietary habits, and pregnancy outcomes. The systematic review has been accepted for publication in the Nutrients scientific journal. On a total of 2401 records identified, we included 29 studies meeting the following criteria: (i) articles published in the English language, describing (ii) observational epidemiological studies, (iii) conducted on pregnant women and/or their offspring, (iv) that assessed maternal and/or neonatal genetic variations, (v) and examined their interaction with maternal dietary habits and/or nutrient intake (vi) in relation to maternal and/or neonatal outcomes.

With respect to maternal outcomes, a total of 6 studies explored the interaction between genetic variants and dietary factors on the risk of gestational diabetes mellitus, while 5 studies explored hypertensive disorders of pregnancy, recurrent spontaneous abortion, recurrent pregnancy loss, iron deficiency anemia, and Gestational Weight Gain.

With respect to neonatal outcomes, a total of 6 studies explored the interaction between genetic variants, dietary factors and anthropometric measures, 8 studies explored abnormal embryonic development, 2 studies preterm birth, and 2 studies other neonatal outcomes. The activities will be pivotal for an in-depth investigation into the interactions among genetic factors, lifestyles, and mother-child outcomes, thereby providing a comprehensive understanding of the underlying mechanisms, in a perspective of precision prevention.

SPOKE 7, TASK 4.2

# Polysaccharide hydrogel containing silver nanoparticles for potential treatment of infected skin ulcers

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Local antibiotics and hydrogels, which resemble the extracellular matrix, are commonly used to treat skin ulcers. However, excessive antibiotic use can lead to microbial adaptation and the emergence of resistant strains. Silver nanoparticles (AgNPs) offer a promising alternative, effectively combating resistant bacteria. In our study, we developed a novel cationic derivative of gellan gum (named GG-EDA-GTMAC) as a precursor for producing injectable hydrogels. In particular, GG-EDA, was dissolved in water in the presence of diethyl amine (DEA), and reacted with glycidyltrimethylammonium chloride (GTMAC) at this temperature for 24 h. The functionalization of the obtained product after purification by dialysis, was investigated through 1H-NMR, and confirmed by TNBS assay. Both spectroscopic (NMR) and colorimetric (TNBS) analysis confirm that the functionalization degree of GG-EDA in GTMAC moieties is 20±5mol%. Therefore, GG-EDA-GTMAC was dissolved in water at 70°C and cooled at 40°C prior to add $AgNO_3$ solution. The obtained sol was irradiated for 30 min with UV light at 366 nm. The presence of reduced silver nanoparticles was investigated by means of UV-Vis, SEM-EDX and XPS analyses. Obtained results demonstrated that AgNPs can be readily produced via UV in situ photoreduction and stabilized within the hydrogel without the need for toxic reducing agents. It is reasonable to suppose that, before UV irradiation, GG carboxyl groups adsorb silver ions and, after irradiation, the permanent cations and residual free amine groups of the derivatives stabilize the silver nanoparticles, preventing their aggregation.
Rheological studies demonstrated the injectability of the hydrogel that makes it easily administrable and suitable for 3D printing. Therefore, this nanocomposite hydrogel can be conveniently injected or printed to achieve specific shapes and dimensions.
Cytocompatibility was investigated and confirmed by MTS test by culturing the hydrogel, containing or not silver nanoparticles, with human dermal fibroblasts. Hydrogels exhibit antimicrobial properties, dependent on the amount of silver payload, against both Gram-negative and Gram-positive bacteria, such as P. Aeruginosa and S. Aureus, as confirmed by colony forming unit (CFU) assay.

SPOKE 6, TASK 3.2

# Evaluation of eosinophil-derived extracellular vesicle secretion and incorporation in tumor cells by flow cytometry and on chip platforms

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Eosinophils (Eo) are major effectors in Th2-related pathologies recently rediscovered for their implications in cancer immunity (1). Like all cells, Eo secrete nanosized (30-200 nm) extracellular vesicles (EV) that release their bioactive molecules (i.e., proteins, lipids, miRNA, mRNA) into receiving cells, affecting their physiology and phenotype (2). The epithelial alarmin IL-33 constitutes a potent stimulus for Eo activation which leads to contact-dependent degranulation and cancer cell death (3, 4). In this study, we generated fluorescent EV by labeling mouse and human eosinophils with Bodipy FL C16 (C16+ EV). By flow cytometry-based enumeration of C16+ EV, we demonstrated that mouse and human Eo activated with IL-33 (Eo33) secrete higher amounts of EV with respect to control eosinophils stimulated with IL-5 (Eo5). The release of Eo33-derived EV (Eo33-EV), but not of Eo5 -derived EV (Eo5-EV), is also increased in presence of tumor cells suggesting that these vesicles may affect tumor cell features once incorporated. EV internalization process within the target cell is a necessary condition to determine their molecular and bioactive cargo release (5, 6). To verify the incorporation of mouse and human fluorescent Eo5 and Eo33-derived EV (C16+Eo5-EV and C16+Eo33-EV, respectively) within target mouse B16.F10 metastatic melanoma cells and human A375P melanoma cells, we co-cultured the immune and tumor cell populations separated by a 0.4 µm-pore size transwell system. The 0.4 µm-pore transwell allows the transfer of fluorescent EV without passage of cells. Through flow cytometry, we measured C16-associated green fluorescence within the tumor cells and found that incorporation of C16+Eo5-EV and C16+Eo33-EV into mouse and human melanoma cells reached > 90% by 18 h. To monitor in real-time by time-lapse fluorescence microscopy the incorporation of fluorescent eosinophil-derived EV within tumor cells, we employed advanced and ad hoc fabricated microfluidic devices. We analyzed over time the transfer of C16+Eo5-EV and C16+Eo33-EV into B16.F10 by culturing BODIPY FL C16-labeled Eo and tumor cells embedded in matrigel and loaded in two different distal side chambers connected by two microchannel arrays and a central fluidic chamber. In these microfluidic systems, EV are able to pass efficiently. The acquisition of C16-associated green fluorescence by B16.F10 melanoma cells revealed an earlier incorporation of C16+Eo33-EV (1 h, with fluorescence peak by 3.4 h) with respect to C16+Eo5-EV (2 h, with fluorescence peak by 4.5 h) in tumor cells. Post fixation DAPI staining of the cells within the microfluidic device at the end of the experiment (18 h) revealed the presence of green fluorescence spots in the cytoplasm of melanoma cells, demonstrating efficient incorporation of C16+Eo5-EV and C16+Eo33-EV by 18 h. We performed similar experiments by using human Eo and A375P melanoma cells. Our findings demonstrate that activation with IL-33 stimulates EV secretion by Eo that are efficiently incorporated into melanoma cells. Fluorescent EV release by Eo and incorporation into target cells can be successfully measured by flow cytometry and tracked in real time by on chip platforms.

SPOKE 7, TASK 1.3

# Activation of ALK signaling pathway induces DNA damage response in colorectal cancer

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In recent years, there have been several attempts to classify colorectal cancer (CRC) into well-defined molecular subgroups that reflect the inherent heterogeneity between patients. Consensus molecular subtypes (CMS) classification successfully achieves this purpose and provides an important tool for personalized medicine. We previously identified an inverse association between high levels of anaplastic lymphoma kinase (ALK) expression and recurrence-free survival, only in the CMS1 subtype, confirmed by a wide array of in vitro and in vivo assays. To support the hypothesis of ALK having a strong implication in CRC patient tumor initiation, we overexpressed ALK receptor in a normal human colon epithelial cell line (NCM460). ALK overexpression was confirmed both in 2D and in 3D spheroid models, cultured in 3D. The role of ALK pathway activation in colonocytes was then investigated by examining its effects on proliferation, survival, migration, and invasion through various 2D and 3D in vitro assays. Immunohistochemistry analysis revealed an increase in the proliferation marker KI67, and a decrease in the E-cadherin (E-CAD) expression in ALK-overexpressing spheroids compared to the control ones suggesting a correlation with tumor metastasis, progression, and invasion. Moreover, we identified a strong correlation between high level of expression of ALK and the downregulation of MSH2, a DNA Mismatch Repair (MMR) protein associated to microsatellite instability, which is typically found in CMS1 patients. Finally, the mass density, size, and weight of in vitro NCM 460 spheroids were analyzed with an emerging microfluidic technology provided by CellDynamics isrl company. Overall, these results suggest that ALK overexpression itself is sufficient to induce aggressive features in normal colonocytes, further supporting the hypothesis that ALK may be an attractive target for CMS1 colorectal cancer therapy.

# Rescuing nonsense in cancer: recovering p53 tumor suppressor gene expression by translational readthrough inducing drugs (TRIDs)

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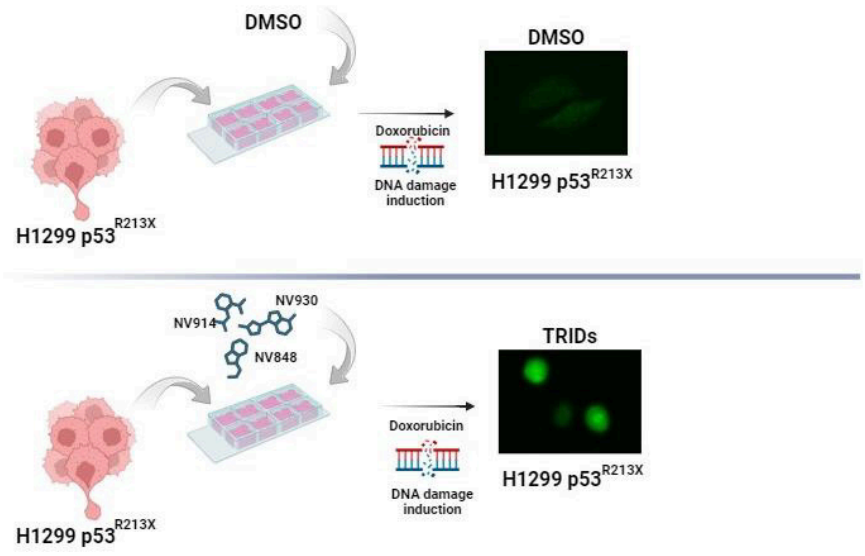
Mutation-based treatments represent a burgeoning frontier in genetic medicine, wherein therapeutic interventions are tailored according to the specific mutation profile of a patient. Recently, considerable attention has been directed towards addressing diseases stemming from premature termination codons (PTCs). A novel class of drugs, known as Translational readthrough-inducing drugs (TRIDs), has emerged with the capacity to facilitate the readthrough of PTCs, thereby reinstating the synthesis of full-length functional proteins. This study focuses on the potential of three newly synthesized TRIDs: NV848, NV914, and NV930, in promoting the production of functional p53 protein from a cDNA sequence carrying a PTC. The investigation involved the treatment of a human cancer cell line (H1299-p53R213X) engineered to harbor a PTC, where significant levels of readthrough were achieved upon treatment with the aforementioned TRIDs. After 24 hours of treatment with TRID molecules, we analyzed the expression of the p53 mRNA by real-time RT-PCR, observing that the treatment induces the stabilization of mutant p53 mRNA, resulting in augmented protein expression that appears functional. The observed upregulation of p53-target genes, after DNA damage induction by Doxorubicin, further corroborated these findings.
These results herald a promising avenue for the development of targeted cancer therapies tailored to address nonsense mutations within tumor suppressor genes. They underscore the feasibility of employing molecules designed to induce stop-codon readthrough as a way to impede tumor proliferation. Moreover, they furnish a rational groundwork for the formulation of novel personalized treatment modalities, thereby enriching the existing repertoire of cancer therapeutics.




Readthrough approach in p53 nonsense mutated cells to rescue protein expression and activity.

# Development of novel bispecific T cell engagers targeting unique tumour antigens for precision cancer immunotherapy

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The development of bispecific antibodies (bsAbs) against tumour antigens is a promising approach in cancer immunotherapy, with a focus on liquid tumours such as acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia (CLL), as well as emerging targets in solid tumours. Bispecific T cell engagers (BsTCEs) are designed to engage CD3 on T cells and specific tumour antigens, enabling T cells to target and kill cancer cells by forming an immune synapse. This study aims to generate and evaluate novel monoclonal antibodies (mAbs) to construct BsTCEs against unique tumour antigens in CLL and AML, and a novel antigen in solid tumours.
Ongoing research has led to the discovery of a large number of antibody-secreting cells (ASCs) in the bone marrow of animals immunised with the selected CLL antigen, which have been isolated and engineered to produce recombinant immunoglobulins. Fifteen antibodies specifically targeting the CLL antigen have been identified to date, five of which recognise native antigens on human MEC cells. Interestingly, these antibodies show no reactivity to MEC cells in which the target antigen has been eliminated using CRISPR/Cas9 technology. Similarly, antibodies against the selected AML antigen have been discovered that show reactivity with the AML cell line HL-60.
Selected potent mAbs will be engineered into bispecific constructs for in vitro and in vivo testing to assess efficacy and safety. In vitro studies will evaluate the ability of BsTCEs to induce T cell cytotoxicity against AML, CLL and solid tumour cells by measuring parameters such as target cell lysis and T cell activation. Cytotoxicity against primary patient tumour cells will also be assessed. In vivo studies using xenograft models will investigate the therapeutic potential of BsTCEs, their biodistribution and impact on tumour burden and survival outcomes. The findings from this study could lead to the advancement of precision therapies for various cancers, providing new avenues for personalised treatment strategies and improved patient outcomes in oncology.

# Prognosis of candidates to lead extraction for cardiac implantable electronic device infections: the role of aetiological agent vs clinical infection pattern

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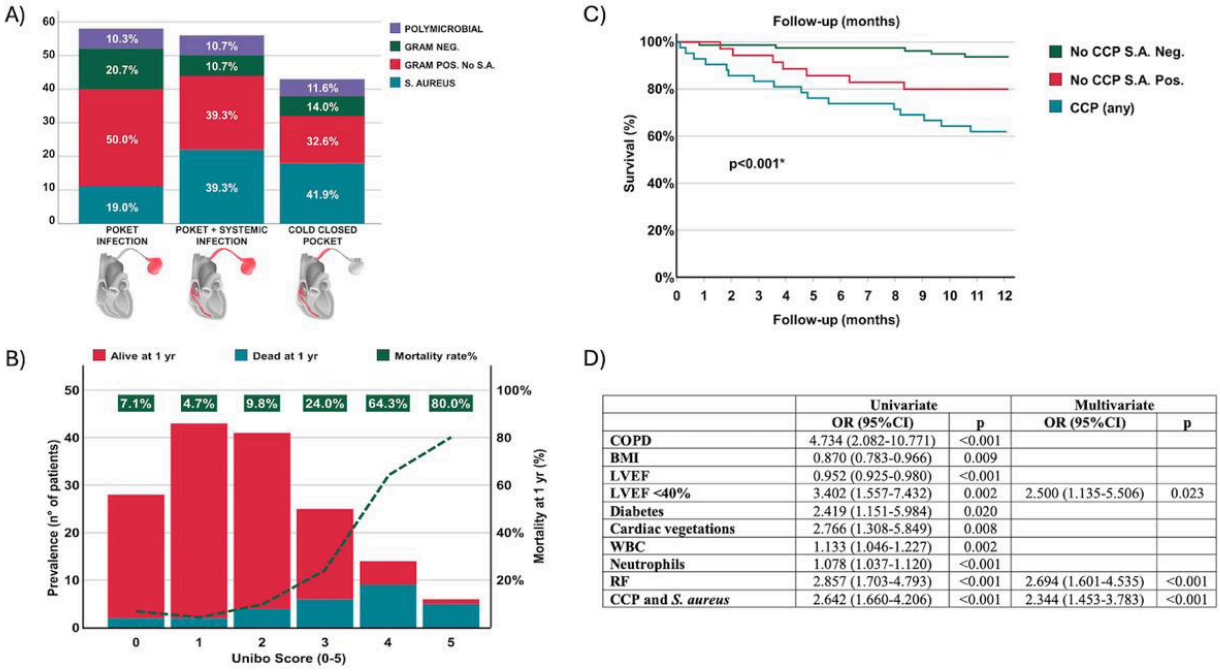
Cardiac implantable electronic devices infections (CIEDI) are associated with poor survival despite the improvement in transvenous lead extraction (TLE). Aetiology and systemic involvement are driving factors of clinical outcomes. The aim of this study was to explore their contribute on overall mortality. A prospective study was performed between 2011 and 2021, including all TLE candidates at our regional referral University hospital for CIEDI with microbiological confirmed aetiology. Considering significant predictors of mortality at multivariate Cox regression analyses, a 5-point BOP2D (S. aureus Bacteria, Cold Closed POcket, renal imPairment, left ventricular Dysfunction) score was developed, and it was validated with a prospective cohort from the Padua University. 157 patients were enrolled (mean age 71.3±12.3 years, 81.5% male). S. aureus was isolated in 32.5% of patients, and it was more associated with valvular heart disease, systemic infection, and chronic kidney disease. CIEDI pattern was associated with 1-year mortality, with a significantly worse outcome in patients with "cold closed pocket" (CCP, infection of transvenous hardware without any clinical or instrumental sign of pocket involvement). The developed BOP2D score presented a 0.807 AUC (95%CI 0.703-0.910, p<0.001) and a good predictive value (OR 2.355, 95%CI 1.754-3.162; p<0.001), and was associated with a progressive increase in mortality with a score >2. The score validation with the registry from the Padua University (135 patients) retrieved a C-statistic of 0.746 (95%CI 0.613-0.879; p=0.002). Both CCP and S. aureus were confirmed as risk factors for mortality in CIEDI patients. This study supports the hypothesis that the infectious process may occur through different mechanisms associated with different infection patterns, and high-risk patients should be considered for specific and aggressive approaches.




| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR (95%CI) | p | OR (95%CI) | p |
| COPD | 4.734 (2.082-10.771) | <0.001 | | |
| BMI | 0.870 (0.783-0.966) | 0.009 | | |
| LVEF | 0.952 (0.925-0.980) | <0.001 | | |
| LVEF <40% | 3.402 (1.557-7.432) | 0.002 | 2.500 (1.135-5.506) | 0.023 |
| Diabetes | 2.419 (1.151-5.984) | 0.020 | | |
| Cardiac vegetations | 2.766 (1.308-5.849) | 0.008 | | |
| WBC | 1.133 (1.046-1.227) | 0.002 | | |
| Neutrophils | 1.078 (1.037-1.120) | <0.001 | | |
| RF | 2.857 (1.703-4.793) | <0.001 | 2.694 (1.601-4.535) | <0.001 |
| CCP and *S. aureus* | 2.642 (1.660-4.206) | <0.001 | 2.344 (1.453-3.783) | <0.001 |

**A)** Distribution of patients according to CIEDI aetiology and pattern. **B)** Mortality considering BOP2D score. **C)** Survival curves considering CCP diagnosis and microbiological aetiology. **D)** Predictors of mortality at Cox regression analysis.

# Computational models for tailoring stroke risk quantification on a patient specific basis

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Stroke risk assessment in patients with cardiovascular conditions is a critical area of research, particularly in understanding the factors that contribute to thrombus formation in the left atrium. This study focuses on integrating additional equations for various protein concentrations into computational fluid dynamics (CFD) simulations to enhance the predictive capabilities for stroke risk. By leveraging the OpenFOAM software and patient-specific anatomical models, we aim to model blood flow dynamics using the Navier-Stokes equations and analyze the distribution of key clotting-related proteins. The ultimate objective is to develop a comprehensive stroke risk index by combining these protein concentrations with hemodynamic parameters, aligning with the principles of personalized medicine.

Our primary objective is to analyze protein concentrations, starting with thrombin and eventually including other clotting-related proteins, in conjunction with established hemodynamic parameters such as wall shear stress, time-averaged wall shear stress, oscillatory shear index, and endothelial cell activation potential. The left atrial appendage is of particular interest due to its complex geometry, lower blood velocity compared to the left atrium chamber and extended blood residence time, which are critical factors in thrombus formation. The use of patient-specific anatomical models enables a personalized assessment of these factors, creating a digital twin of the left atrium for more accurate simulations.

The project is currently focused on integrating the equation for thrombin concentration into the existing CFD framework and exploring appropriate numerical methods and conditions for accurate simulation. Initial steps have involved extensive literature review and preliminary numerical analyses to ensure the robustness of our approach. This groundwork is essential for the accurate simulation of thrombin.

Future work will involve conducting detailed simulations on data acquired in control subjects and paroxysmal and persistent atrial fibrillation patients and validating the derived stroke risk index versus its clinical evaluation based on the CHA2DS2-VASc score. Expanding the study to include additional clotting proteins is also part of future work.

This index ensures a tailored approach to stroke risk assessment and could provide an accurate quantification and stratification of stroke risk, aiding in early diagnosis and prevention strategies within the realm of personalized medicine.

# Electrospun nanofiber mats for microfluidic devices designed for capturing rare cells for diagnostic applications

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Early detection and effective treatment greatly influence survival rates in disease management. Micromanipulation is a reliable method for manually isolating rare cells from biological fluids for molecular or cytogenetic analysis. However, this technique is expensive and time-consuming due to the need for skilled personnel and specialized equipment.
This study aims to improve the efficiency and affordability of diagnosis in hospital settings by developing a device for semi-automated selection of rare cells from biological samples. The device uses electrospun nanofiber mats with a high surface area, functionalized with antibodies to selectively capture target cells based on surface antigens (Fig. 1), intended for use as a substrate in a microfluidic device. Microfluidic systems offer advantages such as micro-scale features that align with many biological systems and laminar flow, which allows for precise fluid delivery, combined with a high surface-to-volume ratio that enhances mass exchange.
Nanofiber mats were obtained from Nylon 6,6 and Polyacrylic Acid (PAA) polymer solutions. The initial phase focused on determining the optimal operating parameters for electrospinning to enhance the mats morphology, mechanical strength, and handling characteristics. Bioconjugation methods using EDC/NHS chemistry were developed to provide the mats with cell-capture capabilities. Fluorescently labeled antibodies were used to assess conjugation success through confocal analysis.
Cell capture tests were performed using mesenchymal stem cells (MSC) as a model on antibody-decorated mats. Optical and confocal microscopy evaluated the capture efficacy, and subsequent release strategies were also investigated. The successful capture of model cells demonstrates the potential of this nanotechnology for developing microfluidic devices for diagnostic purposes.

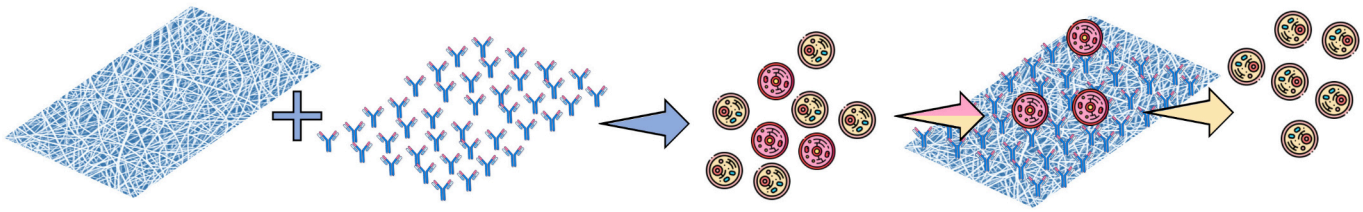

Figure: Schematic representation of mat antibody decoration and specific cells selection.

# A bioinformatics approach to precision medicine

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Over the past decade, the landscape of precision medicine has experienced a notable transformation, driven by the intersection of omics sciences and computational methodologies. This convergence has empowered researchers to explore individual-specific biological markers more deeply, offering the potential for tailored treatments. However, the development of such treatments encompasses several challenges including disease biomarkers identification, integration of heterogeneous data, including multi-omics data (e.g., epigenomics, transcriptomics, and proteomics), spatial tissue organization data, and clinical data. Finally, the accurate identification of potential targets for genome editing and developing patient-specific drugs. At InfOmics Lab, we address these challenges developing innovative and efficient computational solutions for personal genome analysis, patient stratification and drug target prediction. We have created several tools for the analysis of multi-omics data and biomarkers identification, including Stardust (Avesani et al. 2022, GigaScience) for downstream analysis of Spatial Transcriptomics data, EasyCircR (Aparo et al. 2023, biorxiv) for analyzing the post-transcriptional regulatory roles of circular RNAs, DiGAS (Aparo et al. 2023, medrxiv) for single nucleotide polymorphism-based differential allele spectrum analysis between subject conditions and GRAFIMO (Tognon et al, 2021, PLOS Computational Biology) for the identification of individual structural variations inside the genome. Once potential disease biomarkers are identified, we use them as discriminant input features in network-based AI methods for patient stratification, which is crucial for designing personalized and more efficient therapies.

Designing new therapies is another crucial challenge we are tackling by developing a new database for the analysis and prediction of molecular interactions (Viesi et al. 2023, Database), and through the precise identification of potential CRISPR/Cas9 targets using our CRISPRme (Cancellieri et al. 2022, Nature Genetics) tool.

In conclusion, our research develops advanced methods for efficiently analyzing biomedical data, including biological network mining, data integration, omics analysis, pangenome reconstruction, haplotype-aware genome analysis, and patient classification, leveraging machine learning, data science, mathematics, and graph theory, and we aim to combine medical and bioinformatics approaches essential for addressing complex diseases and their associated social consequences. Further details on the lab projects can be found on the InfOmics website: https://infomics.github.io/InfOmics/

# Precision medicine in rare diseases: long-read sequencing to unravel the molecular mechanism of ring 14 syndrome

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Ring14 (r14) syndrome is a rare neurodevelopmental disorder characterised by a complex phenotype including intellectual disability, microcephaly, distinctive facial appearance, eye anomalies and drug-resistant epilepsy. It is caused by the rearrangement of chromosome 14 into a ring-shaped structure originated from two breakpoints that are fused together with terminal deletions. This loss of genetic material does not explain some clinical features: individuals carrying comparable 14q linear deletions don't exhibit epilepsy, thus haploinsufficiency is not the main disease-mechanism.
We aim to genetically characterize the structural complexity of r14 by combining state-of-the-art methods. Thanks to the recently released telomere-to-telomere human genome assembly (T2T-CHM13), where acrocentric chromosomes are fully resolved, we have access to previously inaccessible regions (i.e. 14p). Using long-read sequencing (LRS) we will define the complex structural rearrangement with high-resolution definition of breakpoints.
Data analysis was conducted using Minimap2 for the alignment, Samtools for sorting and indexing, Sniffles2 and Spectre for structural variant calling and Bigclipper for the analysis of clipped reads. We ran the workflow on raw data obtained with Oxford Nanopore Technology (ONT) long-read sequencing on test DNA sample from a patient with r14 syndrome. We accurately identified breakpoints on both the long (approximately Chr14:99,535,000) and short (approximately Chr14:6,376,500) arms of chromosome 14 and highlighted the ring rearrangement represented by soft-clipped reads with supplementary alignments on the other arm of chr14. Using coverage data, we were able to call a terminal deletion on the long arm of chr14.
We will extend the analysis to a cohort of 10 r14 patients and 5 healthy parents (courtesy of Telethon Network of Genetic Biobanks) and integrate LRS results to Genome-wide chromosome conformation capture (Hi-C) and RNA sequencing data. Genetic information will be integrated to clinical features to identify clinical modifiers and prognostic determinants. This novel approach aims to improve genotype-phenotype correlations, and enhance patient care and treatment options for this severe disorder.

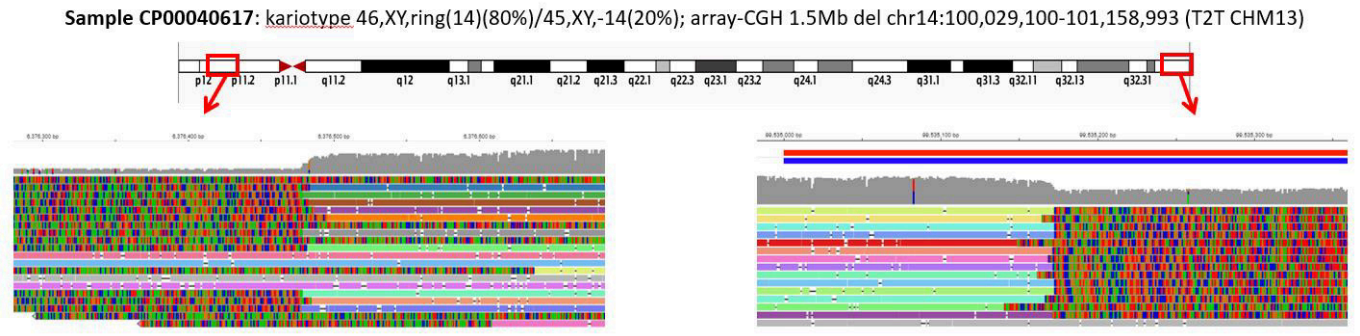


Figure: IGV view of chr14 short and long arms. Reads aligned to T2T-CHM13 reference genome, filtered for mapping quality (MAPQ) > 20. Settings: show coverage; show alignments; show soft clips; color by tag SA (supplementary alignment).

SPOKE 4, TASK 2.3

# Photopharmaceutical strategies with molecular systems, supramolecular constructs and nanomaterials to overcome MDR in cancer cells

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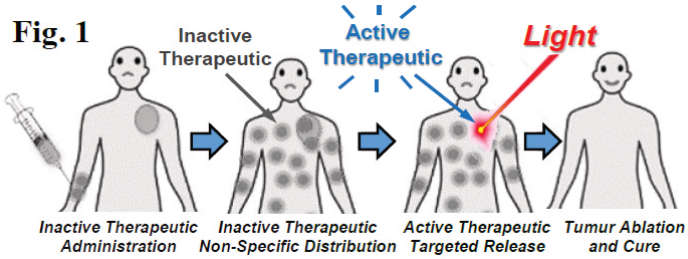
The alarmingly low turnover of new clinically approved chemotherapeutics and the Multi Drug Resistance (MDR) phenomena emerging for drugs actually used, call for an urgent shift of attention to "unconventional" and underexplored therapeutic modalities to tackle cancer diseases. In this frame, photopharmacology is an emerging specialty that promises to revolutionize the therapeutic approach to cancer diseases, allowing more precise and safer therapies for patients. In this approach, light-activatable therapeutics remain inactive and nontoxic once introduced into the human body and only when activated by local irradiation produce tumor-killing species in the diseased area (Fig. 1).
The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as unconventional therapeutic agents has gained an increasing interest in the last years, opening new horizons for the development of innovative therapeutic treatments.
Photodynamic therapy (PDT) is the most promising unconventional approach to cancer treatment already employed in clinic. It primarily relies on the cytotoxic action of the highly reactive singlet oxygen ($^1O_2$). This species is a potent oxidant generated catalytically through energy transfer between the excited triplet state of a photosensitizer (PS) and nearby oxygen molecules.
Recently, photodynamic treatments using nitric oxide (NO) as an unconventional therapeutic, known as NO-PDT, have emerged as a promising area of cancer research. NO plays various roles in bioregulation and can be an effective anticancer agent when produced within the appropriate concentration range. Unlike the catalytic process of $^1O_2$ photogeneration, NO photoproduction involves the light-induced uncaging of NO from scaffolds called NO photodonors.
Both $^1O_2$ and NO are multi-target agents that do not suffer from MDR phenomena. Due to their short lifetimes, their action is confined to short distances from the production site within cells. This confinement helps reducing systemic toxicity, a common issue of many conventional drugs. Additionally, NO photorelease does not require molecular oxygen, allowing NO-PDT to complement PDT under hypoxic conditions, typical of some tumors.
Herein we propose the development of novel light-activatable molecular systems, supramolecular constructs and nanomaterials able to generate individually, sequentially or simultaneously $^1O_2$ and NO with the aim to develop novel innovative photopharmaceutical approaches to cancer treatment.



# Computational prediction and experimental validation of mesothelin-Fn3 binding interface

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Mesothelin (MSLN) is 40 kDa surface glycoprotein bound to the membrane through its C-terminus by a glycosylphosphatidylinositol (GPI) anchor. MSLN is overexpressed in many solid tumors and it is known to interact with the cancer agent CA125/MUC16, promoting cancer cells adhesion and metastasis. Thus, MSLN has been studied as a biomarker for cancer diagnosis and treatment. In fact, MSLN was used as a target of multiple antibody-based strategies, which resulted in limited efficacy, potentially due to antibodies' large size (~140 kDa). To overcome these limitations, we engineered a small scaffold based on the tenth type III domain of human fibronectin (Fn3, 12.8 kDa) to bind MSLN with high affinity in MSLN-positive cell lines (KD=12±4 nM). Fn3 structure is characterized by a His-(6X)-tag at the C-terminus, useful for purification and labelling, and three variable regions (FG, BC and, DE loops), amenable to diversification. Although the MSLN-binding Fn3s were observed to internalize into MSLN-expressing cancer cells and induce apoptosis, MSLN-Fn3 interaction site was poorly explored. Here, we used protein-protein docking and molecular dynamics (MD) simulations to predict MSLN-Fn3 binding site, and to identify the residues involved in the binding interface. We experimentally validated the prediction through a combination of domain-level epitope mapping and yeast surface display. First, Fn3-MSLN binding was predicted by a consensus strategy using three protein-protein docking algorithms (HDOCK, pydock, and ClusPro). Then, MD simulations were carried out using the GROMACS software package and the Amber ff99SB-ILDN force field. To validate the proposed models, full-length MSLN, combinations of or single subdomains (called A, B, C, and D) were expressed on the yeast surface (Figure 1A). Flow cytometry was used to assess MSLN domains expression and to analyse Fn3-MSLN binding. The three docking algorithms predicted Fn3 to bind MSLN in its membrane-distal region (close to the N-terminus) (Figure 1B). The models showed that the Fn3 residues mostly contributing to the interaction are in Fn3 BC and DE loops. Overall, experimental data agreed with the docking prediction. Epitope mapping also showed that MSLN domain B is predominantly involved in the interaction. Interestingly, Fn3-MSLN binding site partially overlaps with a minimal domain on MSLN that interacts with CA125/MUC16, highlighting that Fn3 potentially bocks MUC16-MSLN interaction, possibly affecting cancer progression. Further research will include competition binding assays to test if Fn3 could outcompete MUC16.

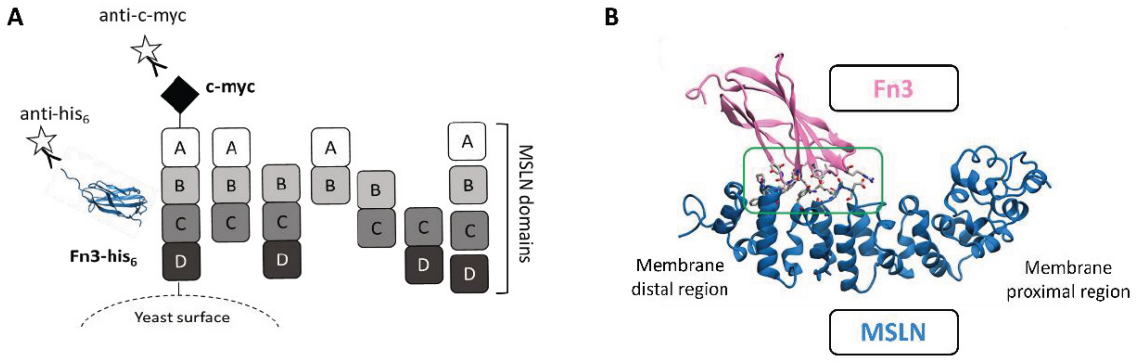


Figure1. (A) MSLN expressed on yeast surface. MSLN domains expression was evaluated through c-myc epitope labelling. Fn3-MSLN binding was analysed using an anti-$His_6$-tag antibody; (B) MSLN-Fn3 binding interface. The residues involved in the binding are highlighted.

SPOKE 3, TASK 3.1 and 3.3

# Data science for health image alignment: a user-friendly open-source ImageJ/Fiji plugin for aligning aultimodality/immunohistochemistry/immunofluorescence 2D microscopy images

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Most of the time, the deep analysis of a biological sample requires the acquisition of images at different time points, using different modalities and/or different stainings. This information gives morphological, functional, and physiological insights, but the acquired images must be aligned to be able to proceed with the co-localisation analysis. Practically speaking, according to Aristotle's principle, "The whole is greater than the sum of its parts", multi-modal image registration is a challenging task that involves fusing complementary signals (Figure 1). In the past few years, several methods for image registration have been described in the literature, but unfortunately, there is not one method that works for all applications. In addition, there is currently no user-friendly solution for aligning images that does not require any computer skills. In this work, DS4H Image Alignment (DS4H-IA), an open-source ImageJ/Fiji plugin for aligning multimodality, immunohistochemistry (IHC), and/or immunofluorescence (IF) 2D microscopy images, designed with the goal of being extremely easy to use, is described. All of the available solutions for aligning 2D microscopy images have also been revised. The DS4H-IA source code; standalone applications for MAC, Linux, and Windows; video tutorials; manual documentation; and sample datasets are publicly available at: www.filippopiccinini.it/DS4H-IA.html

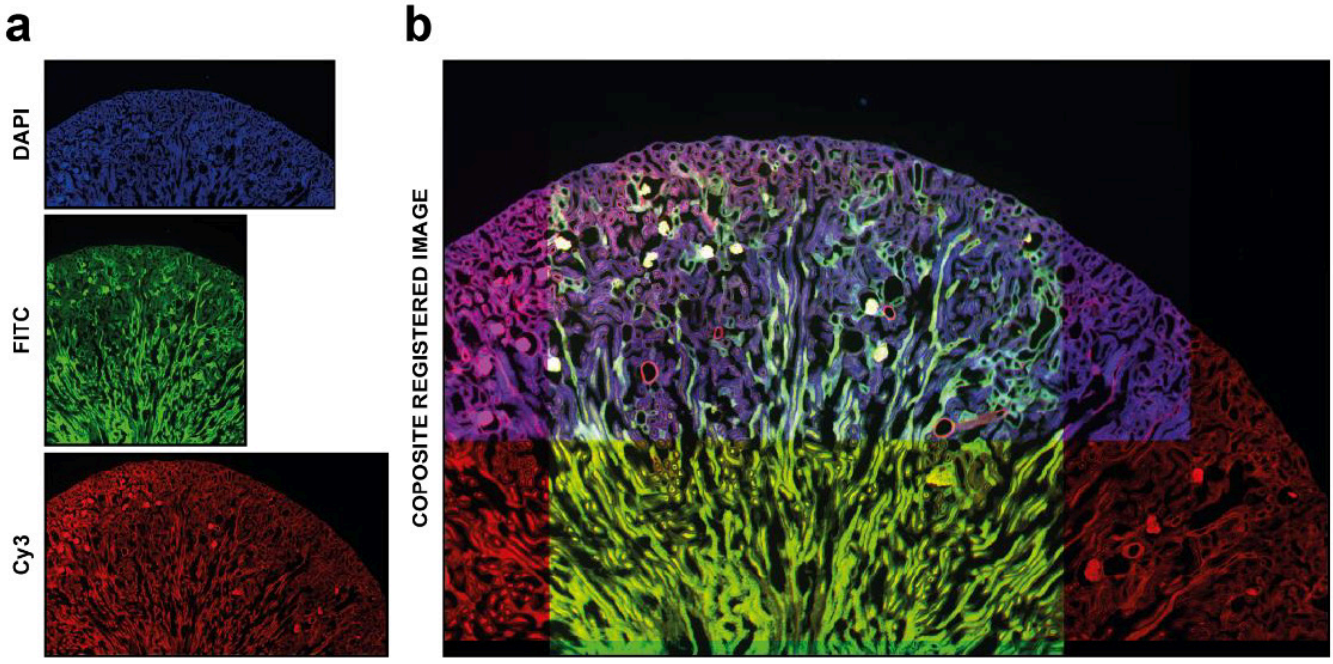


Figure 1: Example of usage of DS4H-IA. (a) Input images referring to a commercial mouse kidney biopsy. From top to bottom: Fluorescence DAPI (nuclear staining), FITC (cytoplasmic staining), and Cy3 (membrane staining) images acquired using a Nikon A1R confocal Microscope equipped with a 20x objective. (b) Example of a common output registered stack.

SPOKE 2, TASK 2.2

# Translational readthrough approach to fight nonsense in cancer: design and synthesis of new trids rescuing P53

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Genetic disorders are caused by mutations that can lead to a nucleotidic modification in DNA sequences, which can involve one or more gene, or abnormalities in chromosome structure. Among different type of mutations, nonsense mutations cause the conversion of an amino-acid coding triplet in a Premature Termination codon (PTC) leading to a decrease in cytosolic mRNA level and a premature termination of the translation with production of truncated and non-functional protein. Nonsense mutation account for the 11% of genetic disease and affect 12% of tumor suppressor genes, among which TP53, one of the most frequently mutated tumor suppressor gene in human tumors. TP53 encode for p53, a transcription factor, known as «the guardian of the genome», that plays different roles in the cell including: antiproliferative activities, DNA repair, apoptosis induction and senescence induction. More than half of cases of all human cancers are characterized by mutations in TP53, in this respect, 10% are nonsense mutations, underscoring the crucial importance of developing new treatment. A prominent strategy in nonsense mutation treatment is based on the pharmacological inducing of translational readthrough (RT) , that leads to the suppression of a stop codon at a post-transcriptional level exploiting molecules called translational readthrough inducing drugs (TRIDs). In this work we identified new chemical scaffolds containing an 1,2,4-triazolic heterocylic core through the development of a new pharmacophore model. After the synthesis of the new compounds we assessed their ability to induce translational readthrough with a preliminary biological screening using the Firefly luciferase assay (FLuc). The best performing molecules were chosen to determine the efficacy in promoting the production of a complete length p53 protein.

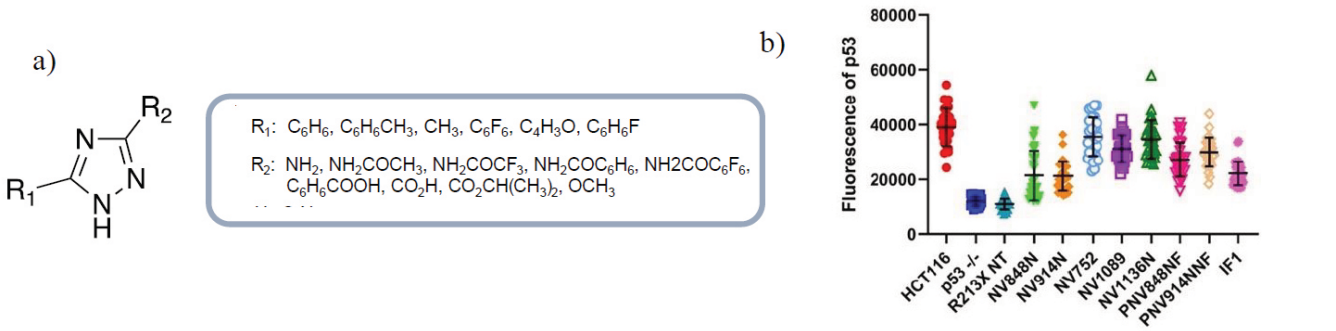


**Fig.1** a) General structure of new hit compounds. b) Quantification of the p53 signals relative to immunofluorescence analysis of H1299 treated with 12μM conc. for each compound for 24h

# Nose-to-brain delivery of nanomedicine to delay cognitive impairment related to metabolic syndrome

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Metabolic syndrome (MetS) as a combination of risk factors (abdominal obesity, dyslipidemia, hypertension, and hyperglycemia) has been widely investigated, focusing on its peripheral effects. Rising evidence suggests that MetS could also increase the risk and progression of mild cognitive impairment (MCI) to dementia. An early intervention in patients with metabolic disorders, in the presence of risk factors, might delay the onset of MCI and its transition to dementia. In the present project, we are focusing on brain targets which are affected by the presence of MetS and whose modulation can provide neuroprotection during metabolic diseases, exerting also control on energy balance. To this aim, several naturally occurring compounds represent good candidates, namely polyphenols such as resveratrol, quercetin and curcumin; flavonoids as genistein, isoliquiritigenin, epigallocatechine gallate, tropoflavin, luteolin, kaempferol; lignans like honokiol; and hormones like melatonin. The clinical application of these molecules is often impaired by their poor pharmacokinetic characteristics, as well as low aqueous solubility, first pass metabolism, and inability to cross the Blood Brain Barrier (BBB). These limitations can be overcome with the use of nanomedicine and the nose-to-brain (N2B) route of administration to reach the brain. Physico-chemical properties of nanomedicines affect their fate after intranasal administration (systemic or direct pathways are involved). With regards to this point, using machine learning we performed a correlation study between the physico-chemical properties (mean particle size, Zeta potential) and drug targeting efficiency percentage (DTE%) and direct transport percentage (DTP%). Results showed that Zeta potential may be more significant than particle size for DTP/DTE predictability. In order to delivery bioactive molecules, with the aim to improve their pharmacokinetics and bioavailability, we developed and evaluated different nanocarriers using a Quality by Design (QbD) approach, as a tool to improve translational research. For this purpose, nanocrystals (NCs), hybrid nanocapsules (hNCs), and polymeric nanoparticles (NPs) were developed, after building the design space through literature search. Fluorescent NPs, starting from PEG-PLGA and PHEA-PLA labelled with a Near Infrared (NIR) probe (DY-700), were further developed as viable tools for optical imaging, with potential applications in theranostics. The expected benefits on using the PHEA-PLA polymer are the possibility of functionalization with a brain targeting peptide, due to the high chemical versatility of such copolymer.
The studied nanoplatforms were deeply characterized from a physico-chemical, technological and morphological point of view. Further in vitro and in vivo studies will be carried out to assess their effectiveness in brain targeting and their pharmacological effects.

SPOKE 6, TASK 4.1

# Interplay of probiotics and metformin in addressing dysbiosis

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Dysbiosis, an imbalance in the gut microbiome, has been linked to a variety of health conditions. In recent years, there has been growing interest in leveraging probiotics and the diabetes medication metformin to help restore gut microbial homeostasis.

Metformin is the first-line pharmacotherapy for treating type 2 diabetes mellitus (T2DM) and is a complex drug with multiple sites of action and mechanisms of action. Physiologically, metformin acts directly or indirectly on the liver to lower glucose production, and on the gut to increase glucose utilization, increase GLP-1 secretion, and alter the gut microbiome composition.

The gut microbiome of individuals taking metformin has shown alterations in gut metabolomics, specifically an increased ability of the gut microbiota to produce the short-chain fatty acids (SCFAs) butyrate and propionate. These microbially-derived metabolites play important roles in glucose homeostasis and metabolic regulation.

This suggests that metformin can have a significant impact on the composition and function of the gut microbiome. Furthermore, an individual's tolerance or intolerance to metformin may be influenced by the unique characteristics of their baseline gut microbial profile and metabolic capabilities. This highlights the importance of considering the gut microbiome when evaluating metformin therapy and its effects.

The mechanisms by which metformin exerts its hypoglycemic effects through modulation of the gut microbiota include: maintaining intestinal barrier integrity, promotion of SCFA production, regulation of bile acid metabolism, and regulating specific bacteria to maintain glucose homeostasis.

Probiotics containing specific bacterial strains have been shown to competitively exclude pathogens, produce antimicrobial compounds, and modulate the host immune system, all of which can help mitigate dysbiosis. Probiotics can also improve intestinal barrier integrity, reduce intestinal permeability, increase luminal SCFA production, and decrease inflammatory markers.

Interestingly, several studies have reported synergistic or additive benefits when probiotics and metformin are used in combination to address dysbiosis. The mechanisms underlying these combined effects likely involve complex interactions between the gut microbiome, host metabolism, and immune function.

Overall, the available evidence suggests that probiotics and metformin hold promise as therapeutic interventions for managing dysbiosis-related conditions. However, further research is needed to fully elucidate the precise mechanisms of action and optimize the clinical application of this gut-modulating approach. Continued investigation in this area may uncover new strategies for restoring gut microbial balance and promoting human health.

# Proton Pump Inhibitors (PPIs) and their impact on the gut microbiota and Small Intestinal Bacterial Overgrowth (SIBO)

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Proton pump inhibitors (PPI) are the most commonly prescribed classes of medications for the treatment of gastric-acid related disorders including non-erosive reflux disease (NERD), peptic ulcer disease (PUD), prevention of nonsteroidal anti-inflammatory drugs (NSAID) associated ulcers, Zollinger-Ellison syndrome (ZES), gastroesophageal reflux disease (GERD), non-ulcer dyspepsia, and Helicobacter pylori eradication therapy. The current approved PPIs available in the United State (U.S) include Omeprazole, Esomeprazole, Lansoprazole, Dexlansoprazole, Pantoprazole, Rabeprazole. PPIs act by decreasing gastric acid secretion in the stomach by targeting the H+/K+ATPase enzyme, known as the proton pump, found in cytoplasmic membranes of parential cells of the stomach. The use of PPIs has been associated with significant changes in the composition of intestinal flora. Several studies have shown that the prolonged usage of PPIs may lead to dysbiosis which is linked to an increased risk of enteric infections, including Clostridium difficile infection, and may contribute to the development of Small Intestinal Bacterial Overgrowth (SIBO). SIBO is a clinical disorder in which the number of bacteria in the intestine is ≥10 5 colony-forming units (c.f.u.)/ml. When SIBO is present, bacterial metabolism produces carbon dioxide, hydrogen, methane, and short chain- fatty acids. These substances can lead to unpleasant symptoms in the abdominal and Gastrointestinal tract, including Loss of appetite, Abdominal pain, Nausea, Bloating, an uncomfortable feeling of fullness after eating, Diarrhea, Unintentional weight loss, Malnutrition, steatorrhea, vitamin deficiencies (B12, D, A, E).

The diagnosis of SIBO can be challenging, with methods including culture of small bowel aspirates and non-invasive breath tests based on the measurement of methane and hydrogen and a variety of molecular assays as a newer diagnostic approach. A variety of SIBO treatments have been proposed recently, involving antibiotics such as Rifaximin, diets, herbal medicine, and probiotics. The majority of probiotic bacteria belong to the Lactobacillus and Bifidobacterium genera.

While the risk of PPIs for the development of SIBO remained equivocal and inconclusive in many individual studies, a relatively recent meta-analysis has shown that PPI therapy is associated with a moderately increased risk of SIBO. Among the 19 eligible studies, seven found a positive association between PPI intake and increased risk for SIBO, whereas the remaining 12 studies did not show a statistically significant relationship. Furthermore, according to another meta-analysis in which a large population of patients was analyzed, only when a highly accurate diagnostic test ( duodenal/jejunal aspirate culture ) was performed, PPI use appeared to increase the risk of SIBO. Therefore, reports regarding the incidence of SIBO in people taking PPI are inconsistence.

| Study | Participants | PPI-treatment | SIBO-prevalence |
|---|---|---|---|
| Revaiah et al.2018 | 91 patients | 6 months | 12/91 – 13% |
| Duran-Rosas et al.2024 | 38 healthy subjects | 7 days | 7.8% |
| Lombardo et al.2010 | 450 patients , 200 GERD | 36 months | 50% |

# Establishing a digital pathology facility

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Digital pathology represents a transformative leap in medical diagnostics, enabling the analysis of histopathological images in a digital format. This innovative approach offers a plethora of advantages, including enhanced diagnostic accuracy, streamlined workflow efficiency, and improved accessibility for second opinions through telepathology. Furthermore, digital pathology facilitates medical and pathological training by providing interactive learning opportunities and expedites scientific research by facilitating the swift retrieval of histopathological aspects. The objective of our project was to establish a digital pathology facility and to share our experience by creating a comprehensive Standard Operating Procedure (SOP). This SOP aimed to seamlessly integrate advanced technology into daily practices, thereby enhancing training effectiveness and supporting research endeavors. The creation of the SOP involved several key components. Firstly, meticulous attention was given to the selection of appropriate technology. This encompassed the procurement of slide scanners meeting specific criteria such as continuous loading capability for uninterrupted scanning, high-resolution capabilities, high-speed scanning functionality, and compatibility with major image management software. Similarly, diagnostic monitors were chosen based on stringent parameters, including high resolution, high refresh rate and precise color representation, to ensure accurate visualization of digital pathology images. Additionally, thorough evaluation of infrastructure requirements was conducted, encompassing storage needs and network connectivity considerations. Subsequently, the SOP delineated detailed processes encompassing equipment installation, software configuration, and day-to-day operations. Simultaneously, tailored training programs were developed to equip staff with the requisite technical and operational competencies essential for utilizing the digital pathology infrastructure effectively. The SOP implementation yielded notable outcomes, including the ability to rapidly share digital images facilitated collaboration among specialists and enhanced training efficacy. Additionally, the digitization of pathology materials expedited research endeavors, with the potential for further advancements through the application of AI-assisted diagnostics and machine learning-based cohort creation for future innovations. Establishing a digital pathology facility signifies a significant milestone in advancing medical diagnostics, training, and research. The SOP serves as a pragmatic roadmap for seamlessly integrating digital technology, thereby unlocking numerous benefits including enhanced efficiency, precision, collaboration, teaching quality, and research support. Moving forward, the implementation of an automated physical archive for case traceability is deemed necessary to complement the digital infrastructure and ensure comprehensive management of pathology materials. Additionally, the need for standardizing the colors of digital slides using appropriate applications has emerged as a crucial consideration, ensuring consistency and accuracy in diagnostic interpretation.
This standardization enhances interoperability between systems and facilitates effective communication among pathologists, further enhancing the utility and reliability of digital pathology platforms. Moreover, the standardization of digital slides also enables reproducibility in artificial intelligence systems, ensuring consistent performance and reliable results in diagnostic processes.

SPOKE 4, TASK 3.1

# Revolutionizing the lung disease therapeutic approaches: Iloprost-loaded particles to treat hyper-inflammation associated with cystic fibrosis

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Based on the well-documented advantages of using inhalable nanomedicine for the local treatment of lung pathologies, this work describes the production of inhalable particles for the administration of Iloprost for the treatment of hyperinflammation associated with cystic fibrosis (CF). CF is an autosomal recessive genetic disorder caused by mutations in the CFTR gene, which encodes a crucial chloride/bicarbonate channel for fluid balance in epithelial cells. Although CF is a disease affecting many organs and tissues, its most severe impact is on the respiratory system, leading to abnormal airway surface liquid, altered mucosal properties, and reduced mucociliary clearance, resulting in chronic infections and hyperinflammation. To date, conventional anti-inflammatory therapies like corticosteroids and non-steroidal drugs are rarely used for CF due to adverse effects and poor penetration through CF mucus. Instead, CFTR modulators, which restore chloride fluxes in bronchial epithelium by correcting and/or potentiating the mutated CFTR protein, have become a focus. Recent findings show that prostacyclin analogues can induce the expression of mutated CFTR protein by increasing cAMP levels in bronchial epithelial cells, thus becoming a new promising approach for CF treatment. Among these, Iloprost, a synthetic prostacyclin approved for pulmonary arterial hypertension, faces challenges with systemic side effects and frequent inhalation treatments. Therefore, encapsulating Iloprost into colloidal carriers can offer significant advantages, including improved stability and ability to modulate its pharmacokinetic and pharmacodynamic drug profile. Here, polymeric nanoparticles (NP_PEG+Ilo) were produced starting from an amphiphilic graft copolymer obtained by functionalization of the α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA) with poly(lactic-co-glycolic acid) (PLGA) and poly(ethylene glycol) (PEG). These nanoparticles showed colloidal size (~150 nm) and negative ζ potential; thanks to the high surface pegylation degree they did not interact with mucins and were able to ensure a sustained-release of the entrapped drug in a simulated physiological fluid. The NP_PEG+Ilo particles were then embedded in mannitol-based microparticles by spray-drying. The resulting Nano-into-Micro (NiM) particles had characteristics suitable for pulmonary administration in CF patients, such as spherical shape, micrometric size (~2 μm), non-interaction with CF artificial mucus components, and the ability to rapidly dissolve and release the NP_PEG+Ilo upon contact with water. Additionally, NP_PEG+Ilo, entrapped into NiM particles, exhibited high cytocompatibility and pronounced anti-inflammatory effect toward CFBE cells overexpressing F508del-CFTR reducing IL1-β, TNF-α, IL-6 and IL-8 gene expression, underscoring the potential of this formulation as a carrier for Iloprost delivery, holding promise for anti-inflammatory therapy in CF patients.

SPOKE 6, TASK 4.1

# Mesothelin-binding Fn3 as a novel therapeutic tool for mesothelioma

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Pleural mesothelioma (PM) is a highly lethal cancer that currently lacks effective therapeutic strategies. One promising target for therapy is mesothelin (MSLN), which is overexpressed on the surface of PM cells. Antibody-drug conjugates that target MSLN have been developed, but their therapeutic efficacy is limited due to their large molecular size, approximately 140 kDa. To address this limitation, smaller scaffolds ranging from 5 to 30 kDa have recently been proposed.

In our study, we focused on evaluating a small scaffold derived from the tenth domain of type III human fibronectin (Fn3), which has a molecular weight of about 11 kDa, for its ability to bind to MSLN. Initially, we generated two cell lines that overexpress MSLN, derived from the PM cell line MSTO-211H. These cell lines were crucial for assessing the binding properties of the Fn3 scaffold. We then performed binding affinity evaluations using flow cytometry and immunofluorescence assays. Flow cytometry analysis indicated that this particular variant of Fn3, designated as Fn3_5.3.2, binds to MSLN with high affinity (approximately 11 nM). Immunofluorescence assays confirmed this binding and provided visual evidence of the cell surface localization of the Fn3-MSLN complexes.

To further investigate the potential applications of this scaffold, we explored whether conjugating Fn3 with chelator molecules, which are essential for radiolabeling, would affect its binding affinity to MSLN. Thus, we conducted similar binding evaluations using a DOTA-GA-conjugated Fn3 variant. Preliminary results from these experiments suggest that the conjugation process has only a minimal effect on the binding affinity of Fn3 to MSLN. However, a complete characterization of the Fn3-DOTA-GA conjugates through mass spectrometry is needed to confirm the degree of Fn3 conjugation.

Overall, our research demonstrates that Fn3_5.3.2 exhibits a strong affinity and specificity for MSLN. This property makes it a promising candidate for delivering cytotoxic molecules, potentially offering a novel therapeutic approach for treating malignant mesothelioma and other cancers that overexpress MSLN. The smaller size of the Fn3 scaffold could also enhance tissue penetration and improve therapeutic outcomes, addressing the limitations posed by larger antibody-drug conjugates.

# Toward a prebiotic formulation to limit growth of Escherichia coli and Enterobacteriaceae

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Enterobacteriaceae is a large family of Gram-negative bacteria that includes many genera, such as Escherichia and Klebsiella. Under normal conditions they represent a small proportion of the human gut microbiota, but in cases of dysbiosis they can be responsible for negative effects on the host's health, due to the presence of opportunistic pathogens. To prevent the overgrowth of harmful Enterobacteriaceae species, the development of novel strategies aimed to the containment of their population can be beneficial to health. In this context, prebiotics offer a promising approach, because they promote the growth and activity of beneficial bacteria such as Bifidobacterium and Lactobacillus, that compete with pathogens for nutrients and produce short-chain fatty acids, lowering the pH of the gut environment, thus creating inhospitable conditions for pathogenic bacteria.
For this study, we tested the effect of different prebiotics on the growth of several strains of Escherichia coli and non-E. coli Enterobacteriaceae (NECE). All strains used were previously isolated from healthy subject feces. Ten strains of E. coli and ten strains of NECE were used, including Klebsiella pneumoniae, Enterobacter kobei, Enterobacter cloacae, Citrobacter amalonaticus, Citrobacter freundii, Cronobacter sakazakii, Raoultella planticola, Hafnia alvei, Klebsiella oxytoca, and Serratia liquefaciens. They were grown aerobically for 24 h at 37 °C in minimal M9 medium supplemented with MgSO4 (0.002 M), yeast extract (0.1 g/L). Prebiotics and their respective monomers (glucose, fructose, galactose, xylose, glucuronic acid, arabinose, maltose, lactose, cellobiose, starch, isomalto-oligosaccharide, inulin, fructo-oligosaccharides, xylo-oligosaccharides, galacto-oligosaccharides, N-Acetyl-D-glucosamine, mucin, arabinogalactan) were utilized as carbon sources. Growth was measured as optical density, using spectrophotometer at 600 nm (OD600). The analyses were performed in triplicate.
The results indicate that the E. coli strains behave similarly. Growth is completely inhibited on substrates such as high molecular weight inulin, arabinogalactan, cellobiose, and xylo-oligosaccharides. However, strains are able to grow on monomers such as xylose, fructose, galactose, arabinose, maltose, and glucose. The 10 species of NECE used exhibit different growth patterns depending on the carbon source, with the exception of high molecular weight inulin and arabinogalactan, which do not allow for the growth of these species.

# Organoids as a model for precision medicine: transcriptome analysis to differentiate malignant pleural mesothelioma from benign reactive mesothelium

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Pleural mesothelioma (PM) is a particularly aggressive cancer caused by asbestos exposure with substantial social impact. This form of neoplasia originates from hyperplasia and metaplasia of mesothelial cells of the pleural cavity. Overlapping morphological features of mesothelial cells make it difficult to discriminate benign reactive mesothelial (RM) lesions from malignant mesotheliomas. Pleural effusion (PE) is the most common initial presentation of PM and occurs in about 70% of patients, predominantly those diagnosed with the epithelioid subtype of PM. PE serves as liquid biopsy, comprising a number of cell types, such as diverse immune cells and stromal cells that accurately reflect the heterogeneity found in PM. In the present study, we used cells derived from PE to establish an ex vivo organoid model that integrate tailored extracellular matrix (ECM) and stromal components, mimicking the disease microenvironment. Patient-derived organoids generate from PM and RM maintained the histological architecture and biomarker expression profile of the tissues from which they were derived. The presence of vimentin further supports their mesenchymal phenotype, while macrophages were an important component of parental tissue and both PM-PDOs and RM-PDOs. Two macrophage population were detected: M1-like macrophages, characterized by CD68 expression negative for CD163, which have been described to generate free radicals and pro-inflammatory mediators, and M2-like macrophages, characterized by both CD68 and CD163 expression, which are considered to promote tumor growth and metastasis. To identify genes involved in malignant tissue, we analyzed the gene expression profile of PM and RM organoids using the RNA-sequencing approach. The whole transcriptome analysis of PM-PDOs and RM-PDOs showed 699 significantly differentially expressed genes (p-FDR <0.05,). Venn diagram indicates that PM and RM share 545 genes (77.9%), while 145 genes are RM-specific (20.8%) and 9 genes are PM-specific (1.3%). By setting PM-PDO with count reads < 10 and RM-PDO with count reads > 30, specific gene signature was established (Figure). This unique gene signature has been proposed as a potential biomarker to differentiate PM from RM and malignancies of metastatic origin, particularly carcinomas.

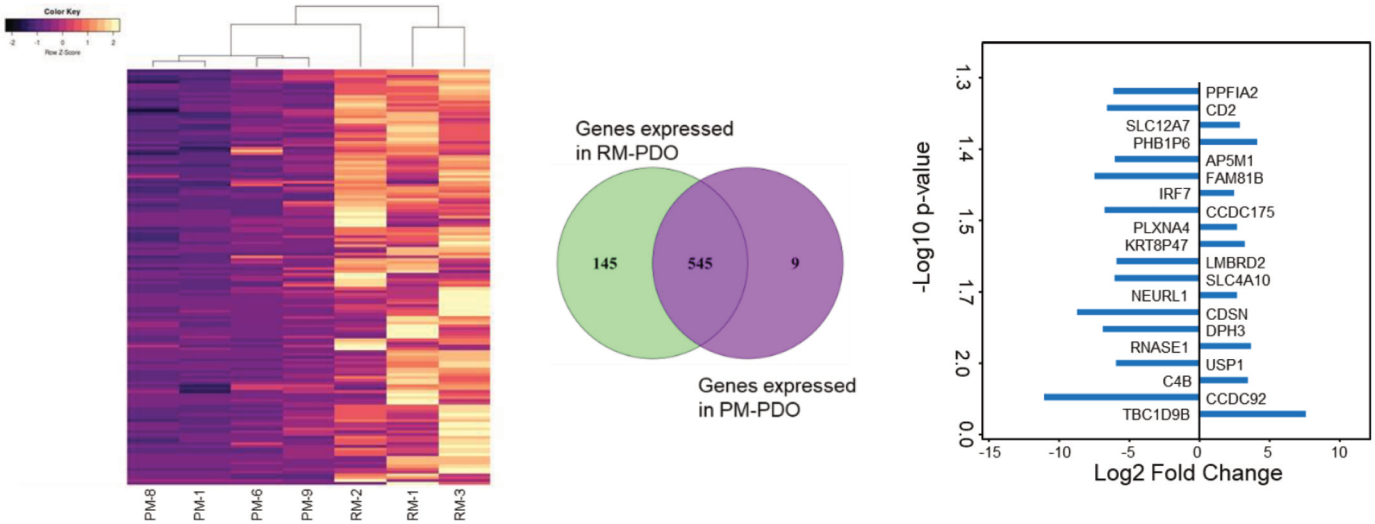


Figure. Transcriptomic analysis of gene expression profile of PM-PDOs and reactive mesothelium patient-derived organoids (RM-PDOs).

SPOKE 7, TASK 1.2

# Diffusion tensor imaging for tissue characterization and risk stratification in atypical ADPKD patients

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Accurate prediction of risk for disease progression is crucial for clinical management of autosomal dominant polycystic kidney disease (ADPKD). The Mayo imaging classification which applies only to ADPKD patients with the typical diffuse cystic disease uses height-adjusted total kidney volume and age to identify patients at the highest risk. In this study we propose the use of magnetic resonance diffusion tensor imaging (DTI) for ADPKD atypical patients' risk classification.

Ten atypical ADPKD patients have been enrolled in the study. DTI acquisition was performed with b-value of 800 s/mm2 and 6/15 different diffusion gradient directions. 3D volumes of interest corresponding to a "cyst-only VOI" and a "parenchyma-only VOI" for each patient were manually drawn. Several diffusion anisotropy indices were computed, and their values were compared between (1) the parenchyma-only VOI and the cyst-only VOI in our population and (2) the corresponding values in healthy kidneys from literature.

All indexes were able to significantly discriminate between cyst and parenchyma, especially the apparent diffusion coefficient (ADC) ($p=7.3x10^{-12}$). Compared to healthy kidneys, in atypical ADPKD patients, parenchymal ADC slightly decreases ($2.11\pm0.0003$ vs. $2.15\pm0.14/2.25\pm0.1$) and parenchymal FA decreases more significantly ($0.184\pm0.098$ vs. $0.28\pm0.05/0.38\pm0.025$). Parenchymal ADC was shown to have the strongest correlation with the serum creatinine and the estimated glomerular filtration rate biomarkers (-0.43 and 0.60, respectively).

DTI has been applied for the first time to ADPKD atypical patients showing its capability of differentiating between cysts and parenchyma. ADC has been shown to be a prognostic biomarker for disease risk stratification.

SPOKE 2, TASK 2.3

# SESSION 3.1

# Gestational diabetes risk and neonatal outcomes: the role of ethnicity

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Over the past decade, migration flows have brought many women of childbearing age to Italy. However, the extent of migration and its impact on the prevalence and outcomes of gestational diabetes (GDM) in Italy are little known.
In this single-center, retrospective, observational study, we evaluated clinical and metabolic data of 4000 pregnant women (81% Italian and 19% non-Italian), who performed selective screening (based on risk factors) for GDM at the Diabetes Clinic of the University Hospital of Pisa, from 2012 to 2022. The proportion of migrant pregnant women referred to our Clinic significantly increased during these ten years (5% in 2012 vs. 25 % in 2022, Chi-square 154.119; p<0.001). In 2022, migrant women mainly were from Albania (18%), Bangladesh (13%), Morocco (12%), Philippines (11%), and Romania (11%). Italian women were older (36 [32-39] vs. 32 [28-36] years, p<0.001), and more frequently at their first pregnancy (52% vs 39%, p<0.001) compared to migrant women. Moreover, assisted reproduction was more common in Italian women (8% vs. 3%, p<0.001), while non-Italian women had higher pre-gestational BMI (24 [22-28] vs. 23 [21-26] kg/m2, p=0.014) and more frequently a history of GDM in a previous pregnancy (13% vs. 9%, p=0.004). The prevalence of GDM was higher in non-Italian women (48% vs. 37%, p<0.001). At multivariate analysis (Table 1), non-Italian women had increased risk of GDM (OR: 1.578 [1.325-1.878], p<0.001), irrespective of other traditional risk factors for GDM. Among women with GDM, metabolic control during pregnancy was worse in migrants (HbA1c in the third trimester: 5.3 [5.1-5.6] vs. 5.2 [5.0-5.4] %, p<0.001). Furthermore, despite similar gestational weight gain (10 [7-13] kg in migrant and 11 [9-14] kg in Italian women) and proportion of women treated with insulin therapy (26% in both groups), non-Italian women more frequently required pre-prandial insulin therapy (34% vs. 21%, p=0.007), either alone or in combination with basal insulin. Neonatal outcomes (preterm delivery, LGA or SGA infants) were comparable between the two groups.
In summary, over the past ten years, the percentage of non-Italian pregnant women referred to our Clinic increased five-fold and, nowadays, one in four women is not Italian. In our study, the risk of GDM was higher in migrants, and migrant women with GDM had worse metabolic control during pregnancy and more frequently required pre-prandial insulin therapy. Close monitoring during gestation, management by a multidisciplinary team and free access to healthcare services may explain the similarity of neonatal outcomes in the two groups.

**Table 1. Univariate and multivariate analysis of risk factors for gestational diabetes (GDM)**

| | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|
| | OR (CI95%) | p-value | OR (CI95%) | p-value |
| Non-Italian | 1.538 (1.311-1.805) | <0.001 | 1.578 (1.325-1.878) | <0.001 |
| Pre-pregnancy BMI | 1.058 (1.044-1.072) | <0.001 | 1.066 (1.051-1.081) | <0.001 |
| Family history for type 2 diabetes | 1.349 (1.182-1.538) | <0.001 | 1.260 (1.098-1.447) | 0.001 |
| Previous GDM | 3.421 (2.731-4.286) | <0.001 | 3.105 (2.464-3.913) | <0.001 |
| Age | 1.016 (1.006-1.026) | 0.001 | 1.021 (1.010-1.032) | <0.001 |
| Assisted reproduction | 0.967 (0.751-1.244) | 0.792 | / | / |

# Clinical Data Repository Next Gen (CDR-NG): AI for more informed and effective research and healthcare, towards precision medicine

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In precision medicine, structured data is essential, yet many current health data remain unstructured. Health data comes from various sources, and integrating them into a system that makes them easily accessible, interoperable, and harmonized in terms of medical terminology is a complex challenge. Additionally, precision medicine requires access to large volumes of data; thus, developing large-scale databases that are both reliable and interoperable presents significant challenges. Engineering is developing CDR-NG, a pivotal tool for precision medicine enabling:
- Proactive data integration and conversion: Allows the proactive integration and conversion of data from various sources, preparing them for future uses, and enriching standard terminologies with proprietary ones leveraging terminology services, thus facilitating standardization, exchange, and interpretation of health data.
- Access to the complete medical history of patients: Enables healthcare providers to access a comprehensive patient's clinical history, even if the original documents are scattered across different locations.
- Advanced full-text search: Supports advanced full-text searches on FHIR and CDA resources, as well as on unstructured data such as PDFs and images, thanks to comprehensive content indexing that includes attributes of FHIR resources and binary data, providing high performance in search and information accessibility.
Furthermore, the CDR-NG features include compliance with regulations (GDPR, ISO27001, IEC81001-5-1), compliance with standards (e.g., IHE-MHD, HL7/FHIR), data versioning, model extensibility. It is cloud-native but can also be provided on-premises. It offers no vendor lock-in: based on open-source software used in their community versions, best of breed technology: the best open-source technologies available (e.g., Spring Boot, HAPI FHIR, Docker, MongoDB, ElasticSearch), and high scalability to manage large volumes of clinical data (big data) effectively. On top of the CDR-NG, two additional tools are built to enable precision medicine:
- A tool for researchers that allows leveraging observational data into the CDR-NG according to OMOP common data model: researchers will be able to specify inclusion and exclusion criteria to define cohorts usable for both retrospective and prospective clinical studies. Data of patients belonging to the cohort will be converted and made available in the OMOP format.
- An AI based tool to support physicians in drafting discharge letters: a dedicated LLM exploits both structured and unstructured data contained in the CDR-NG, for suggesting relevant contents such as a summary of the medical history, risk factors, allergies, list of most significant lab results, admission and discharge diagnoses, and detailed summaries of the clinical hospitalization period at the time of the patient's discharge. The tool may improve the precision, efficiency, and time-saving in drafting medical documentation. It demonstrates how generative AI combined with integrated health data can both improve typical healthcare processes and simplify the identification of data, thus providing an actual example of how CDR-NG can boost the realization of AI solutions contributing to precision medicine.

# Probiotics and ACE inhibitors intervention synergistically mitigate dysbiosis: a systematic review and meta analysis

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Dysbiosis or dysbacteriosis is a state of the gut microbiome leading to an imbalance of the microbiota, alteration in their functional composition and metabolism, as well as a change in the localization of the microbial community. Dysbiosis can be described as a disorder of the gastrointestinal tract. Known causes of dysbiosis include drug use. Angiotensin-converting-enzyme (ACE) inhibitors, which are commonly prescribed for conditions such as hypertension, heart failure, and diabetic nephropathy, can cause dysbiosis and change gut microbiota metabolism. Some probiotics have a positive impact on gut health and work synergistically with ACE inhibitors. This synergistic effect can be helpful in maintaining the microbiome of the gut, which is crucial for the overall health of individuals taking ACE inhibitors to manage conditions like diabetes, hypertension, and kidney disease. Until now, a limited number of studies have investigated the synergistic relationship between probiotics and ACE inhibitors. Therefore, the objective of this systematic review and meta-analysis is to confirm the evidence that probiotics can work synergistically with ACE inhibitors to mitigate dysbiosis.

We aim to identify and analyze variables associated with the synergistic effects of probiotics and ACE inhibitors in reducing dysbiosis, utilizing data from studies conducted between 2010-2024.

This systematic review and meta-analysis were conducted following a predefined protocol registered prior to initiation. A comprehensive search strategy was employed to identify relevant studies, followed by strict study selection criteria to ensure the inclusion of high quality research. Data extraction was performed carefully, ensuring the accurate collection of relevant data points. The risk of bias in the included studies was assessed to guarantee the reliability of our findings. Statistical analyses will be performed to evaluate the synergistic effects of probiotics and ACE inhibitors on dysbiosis. Each step will be thoroughly executed to ensure the robustness and credibility of the findings.

The studies that will be included in this review may demonstrate that the co-administration of probiotics with ACE inhibitors resulted in better outcomes compared to the use of ACE inhibitors alone. This suggests that probiotics can play a crucial role in managing gut health in patients undergoing ACE inhibitor therapy. The findings will confirm the potential of probiotics to work synergistically with ACE inhibitors, providing a promising strategy to combat dysbiosis.

In conclusion, probiotics treatment involving ACE inhibitors may enhance therapeutic outcomes and support gut microbiome health.

# Template for Preparation of One-Page Abstract

G. Migliara [2], V. Baccolini [1], L.M. Siena [1], M. Riccio [1], A. Rosso [3], C. Isonne [1], I. Leone [1], C. Marzuillo [1], C. De Vito [1], G. La Torre [1], P. Villari [1]

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Polygenic Risk Scores (PRS) quantify genetic susceptibility to diseases, promising personalized healthcare. This review explores current evidence regarding the economic evaluation of strategies based on PRS or other polygenic risk stratification approaches, scrutinizing their methodologies. The study protocol was registered in PROSPERO (CRD42023442780). A systematic search in PubMed, Scopus, and Web of Science identified full economic evaluations of intervention based on polygenic risk stratification strategies. The quality of the included articles was assessed using the Drummond checklist. Nineteen articles were included in the analysis, with oncological conditions being the most frequently investigated (13), followed by cardiovascular conditions (3). In nearly 80% of the studies, PRS was employed for screening interventions, with the general population being the primary target in 14 out of the 19 studies. All the economic analysis models investigated cost-utility in terms of quality-adjusted life years (QALYs), with Markov models and microsimulations being the most common structures. The majority of these models were based on simulated cohorts derived mostly from North American or European data, with 9 adopting a healthcare system perspective and 6 a societal perspective. Although delivery strategies for PRS testing were rarely addressed, PRS costs were included in nearly all studies. Indirect costs were examined in fewer than half of the studies. In 12 out of 19 studies, the conclusions claimed the cost utility of PRS involving strategies. Despite the technique's potential, evaluations of various strategies based on PRS approaches yield heterogeneous conclusions regarding cost-utility. Our study highlights the factors contributing to this heterogeneity and underscores the need for further exploration, ideally prioritizing real-world data.