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AIRC FOR YOUNG SCIENTISTS

JUNE 5-6, 2025

Abstract Book

Table of contents

ORAL PRESENTATIONS

Cirino Botta **MFAG - 2020**

University of Palermo

Immune and Microbiota Dysregulation as Early Drivers of Progression from MGUS to Active Multiple Myeloma

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Background and hypothesis

Multiple myeloma (MM) is a malignant plasma cell disorder consistently preceded by asymptomatic precursor stages—monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM). While only 5–10% of MGUS cases progress to MM, reliable predictors of transformation remain lacking. Emerging evidence suggests that progressive immune dysregulation and gut microbiota alterations may play a causal role in disease evolution. We hypothesize that specific immune cell imbalances, cytokine profiles, and microbiota composition are early indicators of progression toward active MM and could serve as actionable biomarkers.

Aims

This study aimed to comprehensively characterize immune and microbial changes across the MGUS–SMM–MM spectrum, with the goal of identifying early determinants of malignant transformation and unveiling novel immunological and microbial targets for risk stratification and therapeutic intervention.

Experimental design

We analyzed peripheral blood and bone marrow samples from 106 patients (13 MGUS, 12 SMM, 63 newly diagnosed MM) using eight multicolor flow cytometry panels to evaluate T, B, NK, and myeloid populations, along with immune checkpoint expression. Cytokine and chemokine levels were assessed in 72 samples from these patients and 4 healthy donors using a 48-plex Luminex assay. Fecal microbiome profiling was performed on samples from 10 MGUS, 15 SMM, and 16 MM patients. Additionally, in a subgroup of 26 patients (distributed across all stages), single-cell RNA sequencing, TCR/BCR repertoire analysis, and surface protein profiling (30 markers) were performed to map transcriptional, clonotypic, and phenotypic alterations at single-cell resolution.

Results

Flow cytometry revealed a progressive increase in CD8+ TEMRA cells, especially CD57+ subsets, alongside a decrease in naïve TIGIT+ and TIGIT+TIM3+ CD8+ T cells. Bone marrow CD4+ T cells showed reduced effector memory subsets and a marked expansion of IL-17-producing (Th17) cells. A significant decline in non-classical HLA-DR+CD11c+ monocytes and mature granulocytes was observed in MM patients. Cytokine profiling indicated a broad suppression of pro-inflammatory and T-cell-supporting cytokines (GM-CSF, IFN-γ, IL-2, IL-10, IL-13, MCP-1) in MM.Fecal microbiome analysis revealed progressive dysbiosis, with increased abundance of Proteobacteria, Streptococcaceae, and Enterobacteriaceae in MM, and a loss of beneficial phyla such as Actinobacteriota and Verrucomicrobiota. MGUS samples retained a eubiotic signature enriched in Lachnospiraceae and Bifidobacteriaceae, while SMM represented an intermediate state.Single-cell multi-omic profiling identified transcriptionally exhausted T cells, expanded clonotypes, and perturbed B-cell maturation pathways in MM, providing mechanistic insight into immune escape.

Conclusions

This integrated immunophenotypic, cytokine, microbiota, and single-cell transcriptomic analysis reveals a coordinated disruption of immune surveillance and gut microbial homeostasis during the transition from MGUS to MM. Our findings support the utility of immune and microbial signatures as early predictors of disease progression and provide a rationale for preemptive immunomodulatory and microbiota-targeted strategies in monoclonal gammopathies.

Enrica Calura

Università degli Studi di Padova

Computational tools to dissect ovarian cancer complexity at single-cell level

High-grade serous ovarian cancer (HGSOC) remains one of the most lethal malignancies affecting women, primarily due to its aggressive progression and late-stage diagnosis. The lack of effective early detection tools results in most patients being diagnosed only after the disease has advanced, significantly limiting treatment options and contributing to poor clinical outcomes. Addressing this urgent need for deeper understanding, our study applies single-cell transcriptomics to unravel the complex biology of HGSOC. We collected and analyzed single-cell RNA sequencing (scRNA-seq) samples obtained from multiple patients across various anatomical sites, both before and after neoadjuvant chemotherapy (NACT). To interpret this high-dimensional data, we developed and applied several computational tools—some of which are currently under active development.

Our first tool stratifies normal and tumor cells based on copy number variation (CNV) profiles inferred from scRNA-seq data. This method combines multiple features—copy number burden, ploidy, CNV signatures, and homologous recombination deficiency—to robustly identify tumor cells and mitigate biases in marker-based classification. We explored the dissemination patterns of ovarian cancer, highlighting self-seeding dynamics and revealing persistent cell states across primary and metastatic sites, as well as treatment-resistant populations. To better characterize these states, we introduced two new R packages: **Signifinder**, which leverages public transcriptional signatures from bulk, single-cell, and spatial transcriptomics to explore intra-tumor heterogeneity, and **Mitology**, designed specifically to assess mitochondrial activity using transcriptomic data. Additionally, we are also developing a new version of our MOSClip software, adapted for single-cell data, to enable topological analysis of signaling pathways in the tumor microenvironment. Together, these tools offer a multi-dimensional perspective on HGSOC, with the potential to improve patient stratification, inform treatment decisions, and drive the development of more effective therapeutic strategies.

Matteo Calvaresi

Alma Mater Studiorum - Università di Bologna

NanoPhages: Engineered Viral Vectors for EGFR-Targeted Photodynamic Therapy

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Background and hypothesis

Photodynamic therapy (PDT) is a clinically approved, minimally invasive procedure for cancer treatments. In PDT, a compound with photosensitizing properties (sensitizer, PS) upon activation by light, generates reactive oxygen species (ROS), responsible for cytotoxicity in cancer cells. The success of PDT is usually limited by: i) poor water solubility and low biocompatibility of the sensitizers ii) lack of selective accumulation of the PS at cancer cells, resulting in unwanted phototoxicity.

Phages are naturally occurring viruses that develop high selectivity for certain bacteria, while they are inactive against eukaryotic cells. The great ease of manipulation of the phage genome, allows to display peptides/antibodies on the phage surface, retargeting them to any type of cell. At the same time hundreds of therapeutic and imaging agents can be conjugated onto the phage capsid, providing a robust and flexible platform for anticancer approaches.

Aims

NanoPhage aims to create an innovative phage-based phototheranostic platform for receptor targeted PDT and imaging.

We developed synthetic methodologies to chemically functionalize the phage capsid with sensitizers/imaging tags and direct this viral vector selectively to cancer cells via phage display of targeting-predatory biomolecules. We targeted EGFR receptors, because they are frequently over-expressed in various cancers.

Experimental design

An orthogonal nanoarchitectonics approach (genetic/chemical) was developed to engineer M13 bacteriophages as phototheranostic platforms.

M13 was genetically refactored to display on the phage tip a peptide or a nanobody to bind EGFR. Using an orthogonal approach to the genetic display, the refactored phages were then chemically modified, conjugating hundreds of sensitizers on the capsid surface.

Results

Flow cytometry and confocal microscopy experiments demonstrated the efficient retargeting of the phages to cancer cells overexpressing EGFR. Upon internalization, the phage conjugates generated intracellularly reactive oxygen species, activated by an ultralow intensity light irradiation. The killing activity of cancer cells was observed at picomolar concentration of the phage vector. The mechanism of cell killing was investigated. The phage-bioconjugates showed a high permeation/phototoxicity also into 3D spheroids and ex vivo samples. The nanosafety of the phototheranostic platform and its imaging/therapeutic performances were preliminary evaluated in vivo using hydra vulgaris and zebrafish has model organisms.

Conclusions

NanoPhage project developed a modular phage carrier for targeted cancer treatment and imaging, promoting the development of a theranostic platform able to simultaneously detect, image and kill cancer cells.

NanoPhage combined the advantages of immunoconjugates anticancer therapies (specific, highly effective, minimally toxic) with the possibility to load orthogonally hundreds of effectors in the viral platform. Soft and penetrating irradiation sources were used to activate the NanoPhages. A double-targeting therapy was developed, exploiting the selectivity of the NanoPhages and the possibility to have a focused irradiation at the desired site of action, lowering the collateral damage to healthy tissues.

Carlo Cosimo Campa **MFAG - 2020**

Italian Institute for Genomic Medicine

Mapping of Rab GTPase pathways in cancer

Background and hypothesis

Rab proteins direct therapeutic targets for antibody-based therapies to the cell membrane and affect their accessibility for antibody binding. Understanding the role of Rab GTPase in cancer is particularly important because dysregulation of Rab GTPase can affect the efficacy of immunotherapies.

Aims

We aim to understand how Rab GTPases functionally interact to control surface expression of therapeutic targets for antibody-based therapies, evaluate the role of differential Rab GTPase expression and organization in cancer cells, identify novel regulators involved in Rab GTPase activity, and explore the potential role of pharmacological inactivation of the Rab GTPase pathway in antibody-based therapies.

Experimental design

We use ad hoc developed computational, imaging and biochemical assays.

Results

We found that both expression and biochemical activity of the Rab GTPases are critical in controlling the cell membrane localization of immunotherapeutic targets.

Conclusions

The Rab GTPase pathway can be pharmacologically modulated to control the efficacy of immunotherapies.

Giulio Caravagna **MFAG - 2020**

University of Trieste

Understanding clonal evolution, timing and plasticity of leukaemias from longitudinal data

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Background and hypothesis

The process of clonal evolution in cancer is challenging to decipher because of the complex interplay of many factors, including somatic lesions that accumulate according to endogenous and exogenous mutational forces, and reversible epigenetic events that trigger plastic phenotypes. These factors drive cancer evolutionary dynamics, but remain hidden inside sequencing data, and require sophisticated computational approaches to be extracted.

Aims

In collaboration with units in Milan and Aviano, we sought to use innovative computational models to study the clonal evolution process under treatment from longitudinal samples of acute myeloid (AML) and chronic lymphocytic (CLL) leukaemia. In AML, we focused on the dynamics of clones that harbour a copy-neutral loss of heterozygosity (CNLOH) of the HLA locus as an immune-evasion mechanism after allogenic transplant. In CLLs, we focused on the dynamics of clones with bimodal expression of CD49d, a prognostic marker of time-to-first treatment and overall survival whose expression increases over time.

Experimental design

In both cases, we used high-resolution (100x) whole-genome sequencing (WGS) of samples collected over time, combined with FACs-sorting, to isolate clonal populations of interest and detect their somatic alterations (pre/post-treatment and sorted for specific markers). We complemented WGS data with other omics to validate signals orthogonally whenever necessary. To extract the clonal evolution signals of interest, we developed new bioinformatics approaches for the molecular profiling of these samples, as well as new analysis approaches that use Poisson processes to time mutagenic events in cancer, and a new theory of population epigenetics to model plasticity and clonal evolution from WGS.

Results

In AMLs, clonal evolution analysis revealed that in 10 of 12 cases, the HLA loss genetic event occurred early after transplant, and was characterised by a mutational signature that we linked to ganciclovir, a treatment that causes DNA double-strand breaks and promotes homologous recombination, the key mechanism underlying HLA loss. In CLLs, clonal evolution analysis revealed that CD49d expression is plastic and changes over time, that it is associated to uneven rates of heritability, and that it can be associated with increased proliferation rates for the CD49d+ component.

Conclusions

We developed new computational tools that could understand the temporal evolution of the disease by combining techniques from mathematical modelling and machine learning. These tools could integrate

clonal evolution signals extracted from longitudinal molecular profiles, recapitulating genetic and epigenetic evolution patterns in two complex leukaemias.

By establishing, for the first time, the time-of-origin of genetic immune evasion in AMLs, and the plasticity of epigenetic evolution in aggressive CLLs, we showed that sophisticated computational approaches can help understand the complex patterns of cancer clonal evolution, offering new tools and techniques to deliver better precision medicine.

Carmine Carbone **MFAG - 2019**

Fondazione Policlinico Gemelli IRRCS, Rome

Molecular Dissection of IPMN Progression Reveals Biomarkers and Therapeutic Vulnerabilities in Pancreatic Cancer Precursors

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Background and hypothesis

Intraductal Papillary Mucinous Neoplasms (IPMNs) are well-established precursor lesions of pancreatic ductal adenocarcinoma (PDAC). However, their clinical management remains challenging due to the lack of reliable molecular markers capable of stratifying patients based on their risk of malignant progression. As a result, some patients undergo unnecessary surgical interventions, while others may miss timely therapeutic opportunities. We hypothesized that combining spatial transcriptomics with high-throughput proteomic profiling could uncover molecular markers and therapeutic vulnerabilities that distinguish indolent IPMNs from those with a higher risk of progression, ultimately enabling more precise and personalized patient care.

Aims

To identify markers associated with different stages of IPMN dysplasia.
To investigate functional pathways driving IPMN progression and assess their potential as therapeutic targets.
To define non-invasive plasma biomarkers capable of stratifying patients by risk, supporting early diagnosis and intervention.

Experimental design

We applied spatial transcriptomics (ST) technologies to two independent cohorts of IPMN patients, using complementary platforms to achieve robust, high-resolution transcriptional mapping of the lesions.
A focused analysis was conducted on 43 IPMN samples (12 with low-grade dysplasia [LGD], 31 with high-grade dysplasia [HGD]) using spatial transcriptomics combined with functional assays in both human and murine pancreatic organoids. Among the enriched pathways, we focused on mucin O-glycosylation and identified GCNT3 as a potential key regulator. GCNT3 was pharmacologically inhibited using talniflumate, and its effects on mucin expression, T cell activation, and immune recognition were evaluated. In vivo, the therapeutic impact of talniflumate, both alone and in combination with chemotherapy, was tested in preclinical models.
Simultaneously, we performed high-throughput proteomic profiling on plasma samples from patients under active surveillance, as well as from those with invasive or PDAC-associated IPMN. Proteins of interest were further validated in tissue using spatial transcriptomics, immunohistochemistry, and ELISA.

Results

Our spatial transcriptomic analysis revealed subtype-specific transcriptional markers across the IPMN progression spectrum. HOXB3 and ZNF117 were associated with low-grade dysplasia, SPDEF and gastric neck cell markers with borderline lesions, while NKX6-2 and gastric isthmus cell markers were enriched in

high-grade gastric IPMN, along with transcriptional signatures of TNFα and MYC activation. These findings improve our understanding of the molecular trajectories of IPMN and their transition toward malignancy

Functional studies identified a significant upregulation of the mucin O-glycosylation pathway during progression, with GCNT3 emerging as a central mediator. Inhibition of GCNT3 with talniflumate led to decreased mucin production and increased T cell activation in both human and mouse organoid models. In vivo, talniflumate synergized with chemotherapy, enhancing immune infiltration in tumor tissue.

Plasma proteomic profiling revealed a distinct circulating metabolic signature capable of discriminating indolent IPMN (under surveillance for more than 7 years) from invasive and PDAC-associated IPMN. This signature was confirmed in tissue and further validated by classical ELISA assays, supporting its translational value.

Conclusions

Together, these findings offer a comprehensive molecular map of IPMN progression, highlighting key genes and pathways that define transitions from indolence to malignancy. We identify GCNT3 as a promising therapeutic target whose inhibition enhances anti-tumor immunity, particularly when combined with chemotherapy. Additionally, our discovery of a non-invasive plasma protein signature holds significant clinical potential for early risk stratification.

Overall, this integrated approach advances the molecular understanding of IPMN evolution and paves the way for improved clinical management of pancreatic cancer precursors through targeted therapies and personalized biomarker-driven surveillance strategies.

Eros Di Giorgio **MFAG - 2020**

Università di Udine

Super-enhancers reorganization controls re-sensitization of oxaliplatin-resistant FBXW7-mutated colorectal cancer

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Background and hypothesis

Epigenetic plasticity and large-scale chromatin remodeling characterize tumor evolution and the emergence of subclones resistant to conventional therapies. Catalytically inactive class IIa HDACs (HDAC4, HDAC5, HDAC7, HDAC9) control the targeted recruitment of chromatin remodeling complexes, making them attractive therapeutic targets in oncology.

Aims

The aim of this project is to identify a novel druggable therapeutic target to achieve oxaliplatin re-sensitization in refractory colorectal cancer.

Experimental design

Cancer cell lines, patients' derived organoids and murine models were adopted to investigate the mechanisms of platinum resistance and to test new epigenetic drugs to achieve re-sensitization.

Results

In this study, we found that HDAC4 is proteasomal degraded in cancer cells impaired in DNA repair by homologous recombination and after oxaliplatin treatment. Genetic screening identified FBXW7 as the E3 ligase responsible for HDAC4 degradation. FBXW7 loss-of-function mutations are frequently found in patients with colorectal cancer (CRC) and associated with the development of resistance to OXPT. Forced degradation of Class IIa HDACs using a PROTAC-based compound restored OXPT sensitivity in FBXW7-mutated CRC cells, patient-derived organoids (PDOs) and in mice.

Mechanistically, removal of HDAC4 in FBXW7-mutated CRC cells and PDOs treated with OXPT recreated an epigenetic state comparable to OXPT-sensitive cells. Furthermore, patient profiling based on the epigenetic state of the super-enhancers controlled by HDAC4 successfully identifies a priori CRC patients resistant to platinum.

Conclusions

This study supports HDAC4 as a key mediator of oxaliplatin resistance in FBXW7-mutated CRC and underlines the remodeling of a well-defined repertoire of super-enhancers as part of the process of re-sensitization.

Gabriele D'Uva

Istituto Nazionale di Biostrutture e Biosistemi

Dissecting the cross-regulation among ERBB receptors in breast cancers

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Background and hypothesis

The ERBB family of receptor tyrosine kinases plays a critical role in breast cancer biology. While ERBB2 (HER2) amplification has led to successful targeted therapies in HER2-positive tumors, EGFR, frequently overexpressed in basal-like and triple-negative breast cancers (TNBCs), has not yielded similar therapeutic benefits. This disparity suggests a limited understanding of ERBB receptor interplay in aggressive breast cancer subtypes. In particular, the relationship among ERBB receptors, the tumor microenvironment, and treatment resistance in basal-like/TNBC tumors remains poorly defined.

Aims

We aimed to correlate ERBB receptor expression with clinical outcomes across breast cancer subtypes and to assess changes during metastatic progression. We further investigated how ERBB ligand availability and loss of adhesion influence ERBB receptor expression, heterodimerization, and downstream signaling. Particular attention was paid to how these factors impact phenotypes such as proliferation, motility, anchorage-independent growth, and chemotherapy resistance.

Experimental design

We analyzed clinical datasets to correlate ERBB receptor expression with relapse-free survival and metastatic progression. In vitro, we used basal-like and HER2+ breast cancer models, with ERBB2 knockout or overexpression. Cells were exposed to ERBB-specific ligands (EGF, NRG1, NRG4) under acute and/or chronic conditions. We assessed receptor crosstalk, signaling outcomes, phenotypic behaviors, and transcriptional output.

Results

Transcriptomic analyses revealed subtype-specific associations between ERBB expression and clinical outcome. In TNBC, elevated ERBB3 levels correlated with poor prognosis, despite overall low ERBB3 expression. NRG1 activated the ERBB3/ERBB2 axis, promoting anchorage-independent growth without affecting adherent proliferation or motility, revealing a potential therapeutic vulnerability (Miano… and D'Uva, Cancers 2022).

Conversely, ERBB4 expression correlated with favorable prognosis in HER2-enriched and luminal A tumors, and its levels were lower in metastases. In vitro, ERBB4 activation by NRG4 potentiated anti-ERBB2 therapy effects, suggesting a tumor-suppressive role and a possible combinatorial strategy using ERBB4 ligands (Miano… and D'Uva, Frontiers in Oncology 2022).

In basal-like/TNBC, EGFR expression paradoxically associated with improved relapse-free survival and EGFR levels dropped in metastases, suggesting early tumor-suppressive activity (unpublished data). Consistently, preliminary data indicate that sustained exposure to high-dose EGF downregulates ERBB2 expression and attenuates oncogenic traits in vitro, supporting a potential tumor-suppressive role for EGFR in ligand-rich environments.

Further unexpected preliminary data show that chronic EGF deprivation, ERBB2 supported cell survival by promoting a quiescent-like state. This adaptive plasticity may facilitate tumor dormancy and chemoresistance in HER2-expressing cancers.

Conclusions

Our findings reveal dynamic, context-dependent cross-regulation among ERBB receptors in breast cancer. ERBB2 and ERBB3 cooperate to promote oncogenic traits in TNBC, while ERBB4 shows tumor-suppressive potential. EGFR may act as a context-dependent tumor modulator. These insights align with emerging evidence supporting a role for ERBB2 in HER2-low cancers and advocate for expanding ERBB2-targeted strategies to include HER2-low tumors, including basal-like/TNBC, broadening the therapeutic window for aggressive breast cancer subtypes.

Giovanni Germano **MFAG - 2020**

IFOM ETS

Leveraging DNA Damage Response Pathways to Identify Therapeutic Opportunities in Cancer

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Background and hypothesis

Defects in DNA replication fidelity drive tumorigenesis. The mismatch repair (MMR) system, responsible for correcting base mismatches and small insertion/deletion loops during DNA synthesis, is inactivated in approximately 20% of cancers. MMR-deficient (MMRd) tumors display distinct clinical features, including early onset, metastatic potential, improved prognosis, and remarkable sensitivity to immune checkpoint blockade (ICB). We have previously shown that these traits are linked to elevated somatic mutation rates and the consequent accumulation of neoantigens. In parallel, mass spectrometry-based peptidomics has revealed the presence of MHC class I-associated peptides (MAPs) derived from non-coding genomic regions. Based on this, we hypothesize that non-coding DNA plays a significant role in shaping the immunogenicity of MMRd tumors.

Aims

The project aims to investigate the contribution of non-coding genomic alterations to neoantigen production in MMRd tumors and to identify potential biomarkers of ICB responsiveness.

Experimental design

We utilized murine colorectal cancer (CRC) models with genetic inactivation of the MMR gene Mlh1. Isogenic CT26 cell lines, Mlh1+/+ (MMRp) and Mlh1−/− (MMRd), were subcutaneously injected into both immunocompetent and immunocompromised mice. Whole-genome sequencing (WGS) and RNA sequencing (RNA-seq) were performed on tumor samples collected before and after engraftment. To characterize the immunogenic landscape, custom six-frame peptide databases were generated from MMRp and MMRd transcriptomes. Peptides selectively lost following injection into immunocompetent hosts were presumed to undergo immune editing and were compiled into a dedicated database. Finally, liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, integrated with matched transcriptomic and genomic datasets, was employed to trace the genomic origin of these immune-edited MHC class I-associated peptides (MAPs)

Results

WGS analyses revealed an unbalanced distribution of immune edited alterations across the genome in Mlh1-/- cells grown in immunocompetent mice. Specifically, untranslated (UTR) and coding regions exhibited the largest fraction of immune edited mutations. Moreover, the integrated computational and LC-MS/MS analyses revealed that MAPs originated mainly from atypical translational events in both MMRp and MMRd tumor cells. On the contrary, Mlh1-/- cells showed a strikingly different repertoire of mutated MAPs, mainly derived from UTRs and out-of-frame translation of coding regions.

Conclusions

Multiple lines of evidence indicate that neoantigens can arise from unconventional alterations across the genome. Our findings show that, compared to MMRp CRC, MMRd tumors generate a significantly greater number of non-canonical, mutated peptides capable of binding MHC class I. These results underscore the importance of comprehensively characterizing the neoepitope repertoire in MMRd tumors to uncover novel therapeutic targets and better understand the determinants of tumor immunogenicity.

Simona Giunta **Start-Up - 2020**

University of Rome La Sapienza

Centromeres in Cancer: from black boxes to biomarkers

Background and hypothesis

When I started the AIRC Start-Up grant in 2021, no complete human sequences of centromeres were available. Centromeres are essential genomic loci that mediate chromosome segregation. Alterations in chromosome dynamics and stability underlie majority of human cancer, yet the involvement of centromere mutations as driver in tumor is unknown.

Aims

The AIRC project aims to understand the function and molecular network associated with human centromeric DNA, and to uncover the association with malignant transformations.

Experimental design

To understand the role of centromere instability in human tumors, we have devised novel cutting edge experimental and computational approaches. Ranging from super-resolution and quantitative microscopy, to the latest long-reads third generation sequencing technologies, we applied genome engineering of human cell lines to simulate centromere instability and tumor development in culture. In parallel, we have developed two new computational pipelines to decipher centromeres and repetitive DNA: ReaCh - Repeat Enrichment Analysis of ChIP-seq datasets, and GCP - Genomic Centromere Pipeline, which uncovered an innovative way to barcode centromere for large scale genomic and diagnostic studies.

Results

In the past 4 years, the Giunta Laboratory has:

- Uncovered centromere instability as a major source of physiological and pathological variation in human DNA.
- Super-resolution structure of the centromeric DNA in a variety of cancer and non-cancer cell lines (Di Tommaso et al., 2023; Di Tommaso & Giunta, 2024).
- Identified the tumor sub-type that have mutational signatures within centromeres and which are early events in driving these cancers (Spurio, et al. In prep.).
- Designed genome engineering screen to understand sources of and responses to centromere mutations.
- Discovered novel conserved biomarkers within human centromere that enable rapid and scalable characterization of genomic structure across species and in disease state (Corda & Giunta, accepted).
- Conceived the human centeny map that leverages non-coding centromeric elements for synteny-like comparison across genomes (Corda & Giunta, accepted) and characterize the presence of ecto-centromeric sites outside of the primary constriction (Corda, Saggese & Giunta, in submission).
- Assembled a new human reference genome as one of the first chromosome-level diploid assemblies and the first of an experimentally-relevant cell line, of the non-cancer cell line RPE-1. The RPE1v1.1 has been used by our lab to identify high-precision phased epigenetic landscaped at the centromere (Corda et al., under revision), but will also be used thousands of laboratories worldwide to advance scientific research using the

RPE-1 cell line (Volpe, Colantoni, et al., under revision; Volpe et al, BioRxiv 2023).

Conclusions

Our work in the past four years has shed light to some of the most complex and long-standing dark regions of the human genome, the centromeres. Through the use of advanced methodologies and cutting-edge sequencing algorithms, we were able to unravel the DNA, structure and changes within centromeres, mine fine details of centromeric repeats, understand their chromosome-specific architecture, and design barcodes that can be used as anchors for genomic and centromere comparison across species and in disease states. Our ongoing work will address the functional mechanism(s) through which centromere instability acts as a driver in tumor formation, and in cancer susceptibility at population level.

Paolo Grumati **MFAG - 2020**

TIGEM

FAM134B in colorectal cancer progression: ER-phagy and calcium homeostasis

Background and hypothesis

Tumor cells are exposed to intrinsic and extrinsic stress elements that alter protein homeostasis, thus inducing an ER stress response, which is involved in the survival and adaptation of cancer cells. Therefore, ER stress and UPR attract much attention as putative therapeutic targets for cancer treat-ments. Autophagy activation is a well-known biological response to ER stress and UPR induction. In cancer, autophagy acts as a bimodal process, whose functions strictly depend on cancer type, onto-genesis and progression. Autophagy can promote cancer progression helping cells to survive stresses or induce cytotoxicity and cell death. The involvement of ER-phagy receptor FAM134B in cancer was described in esophageal squamous cell carcinoma (ESCC) and in colorectal cancer (CRC), where several pathological mutations have been characterised. However, the molecular mechanisms underlying its role remain unclear.

Aims

This proposal is aimed at unraveling the molecular mechanisms behind the tumorigenic role of autophagy with a particular focus on ER-phagy and its receptor FAM134B. The research is focused to further biochemically characterize FAM134B and identify the molecular mechanisms that determines its peculiar tumorigenic behavior. I focus on colon carcinoma where FAM134B was described as a tumor suppressor. The obtained results will help to understand FAM134B double role in cancer and will open new perspectives for modulating ER-phagy as therapeutically approach. More important, the obtained data will help to improve medication of cancer patients.

Experimental design

We employed colon cancer cell lines that resemble different cancer stages: CaCo2, SW480, SW48 and HCT116. As not tumoral control, we used CDD cells. We performed full proteome analysis, cell proliferation and cell survival assays as well as Ca2+ flux studies. These experiments were performed in the parental cell lines and in the FAM134B knockout cells that we generated through the CRISPR-Cas9 system. Moreover, we generated stable cell lines, expressing Spilt-GFP-tag FAM134B, to identify FAM134B interactors in the different contexts. Finally, we investigated spheroid formation in parental and FAM134B knockout cells.

Results

We described the proteome landscape of several colon cancer cells and identified how autophagy and calcium signalling are deregulated in different cells resembling different cancer stages.

We focused our attention on the selective autophagy of the endoplasmic reticulum and its receptor FAM134B that is unregulated in colon cancer cells. Over-expression of FAM134B is followed by an abnormal ER-phagy flux in these cells. We analysed the interactome profiles of FAM134B in the CRC. We noticed a positive correlation between the number of calcium related proteins and the aggressiveness of the cancer cells. Analysis of endoplasmic calcium homeostasis confirmed that the presence of FAM134B influences the

release of Ca2+ from the ER. We further investigated the effect of the Ca2+ imbalance and we could observe how FAM134B presences influence Ca2+ release and consequently the response of cancer cells to apoptosis and spheroid formation.

Conclusions

From our studies we identify a new function of FAM134B protein, which not only regulates the ER-phagy flux but also modulate endoplasmic reticulum Ca2+ homeostasis. This function seems to influence the response of colon cancer cells to stress stimuli and apoptosis. Therefore, targeting FAM134B could be a valuable tool to induce Ca2+ mediated apoptosis in colon cancer cells.

Margarita Kirienko **MFAG - 2020**

Fondazione IRCCS Istituto dei Tumori di Milano

Treatment efficacy and DNA damage induced by [161Tb]Tb-PSMA I&T vs [177Lu]Lu-PSMA I&T in prostate cancer cell lines

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Background and hypothesis

[177Lu]Lu-PSMA is an effective treatment for patients affected by metastatic castration-resistant prostate cancer (mCRPC). However, up to 30% of patients don't respond and progression is inevitable. 161Tb has physical properties that indicate it could be superior to 177Lu.

Aims

Aim of the present study was to evaluate and compare the treatment efficacy and the DNA damage induced by [161Tb]Tb-PSMA-I&T and [177Lu]Lu-PSMA-I&T in human prostate cancer cell lines. Specifically, we evaluated cell proliferation and the the phosphorylation of the H2AX histone (gamma-H2AX) as a readout of induction of DNA double strand breaks (DSB) by immunofluorescence.

Experimental design

PSMA-I&T was labelled with 0.5-2.0GBq of [177Lu]LuCl3 or [161Tb]TbCl3 using a ratio of 60-80MBq of radionuclide per nmol of precursor. Both [177Lu]Lu- and [161Tb]Tb-PSMA-I&T showed a radiochemical purity >98% by iTLC and >97% by HPLC. We set up in vitro pulse chase experiments utilizing LNCaP and PC3 cell lines, expressing or not experissing PSMA, respectively. Cells were treated for 4h with either [161Tb]Tb-PSMA-I&T or [177Lu]Lu-PSMA-I&T, at different activity concentrations (10, 5, 1, or 0.5MBq/ml), and then, after washing, allowed to grow for 7 days. To analyse the DNA damage, LNCaP and PC3 cells (2x105) were treated for 4h with either [161Tb]Tb-PSMA-I&T or [177Lu]Lu-PSMA-I&T (either 10 or 5MBq/ml). As a positive control for the immunofluorescence for □-H2AX, we used LNCaP cells treated with 5mM of doxorubicin for 24h. Slides were washed and stained with primary anti-phospo-Histone H2AX antibody (dilution 1:400) overnight at 4°C. After washing, alexa fluor 488-conjugated chicken anti-rabbit secondary antibody (dilution 1:300) was added for 2 h. The staining intensity was quantified using the ImageJ 2 software. All experiments were performed at least twice. Pools of biological replicates obtained in all the independent experiments were performed and statistical analysis was run considering all the samples. One-way ANOVA was performed separately between LNCaP and PC3 groups.

Results

Both radiopharmaceuticals were able to restrain the proliferation of LNCaP cells with a dose-dependent effect. [161Tb]Tb-PSMA-I&T was superior than [177Lu]Lu-PSMA-I&T at the highest activity concentration (10MBq/ml). We found the highest staining intensity in LNCaP cells treated with 10MBq/ml of [161Tb]Tb-PSMA-I&T. Albeit the difference was not statistically significant, data clearly showed a trend of increased DNA DSB induction in LNCaP cells treated with 10MBq/ml of [161Tb]Tb-PSMA-I&T compared to LNCaP cells treated with 10MBq of [177Lu]Lu-PSMA-I&T. Notably, in PC3 cells the gamma-H2AX staining intensity remained low with no significant variations between control and treated cells.

Conclusions

[161Tb]Tb-PSMA-I&T resulted superior in inhibiting prostate cancer cell proliferation compared to [177Lu]Lu-PSMA-I&T. Our results suggest DNA DSBs as factors reflecting higher efficacy of Terbium-161 labelled radiopharmaceuticals compared to those labelled with Lutetium-177.

Luca Lanzanò **MFAG - 2018**

University of Catania

Interplay between oncogene-induced genomic damage and chromatin organization studied by advanced fluorescence microscopy

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Background and hypothesis

Activation of oncogenes may trigger the generation of genomic damage through alterations of the dynamics of DNA replication, transcription and DNA damage response (DDR). However, despite their potential importance in cancer development, the molecular mechanisms that underlie oncogene-induced genomic damage are still poorly understood. Our core hypothesis is that oncogene activation induces an alteration in the spatio-temporal organization of DNA replication and/or transcription, favoring the occurrence of replication and transcription stress at specific sites in the genome.

Aims

The main goal is to elucidate, by means of advanced optical microscopy (including confocal and super-resolution imaging), molecular details of the origin and evolution of oncogene-induced genomic damage. Specifically, we aim to visualize if the spatio-temporal organizations of transcription and replication sites are altered following oncogene activation and if these alterations correspond to an increase of genomic damage. In addition, we aim to visualize the role of alterations of the DDR (e.g. effect of PARP inhibitors) on the evolution of oncogene-induced genomic damage.

Experimental design

We apply advanced confocal and super-resolution imaging in a model of PML-RARα oncogene activation (U937-PR9 cell line), to visualize alterations of chromatin organization in intact cell nuclei. We setup a protocol for fast live cell imaging to study the effects of PARP inhibitors on chromatin re-organization during DNA damage induction.

Results

We find that activation of the PMLRARα oncogene induces alterations in the coordination of replication and transcription and higher occurrence of DNA double strand breaks, during the S phase of the cell cycle. Oncogene-induced DNA damage is preferentially located in regions of lower chromatin density. Inhibition of PARP1 by PARP inhibitors increases oncogene-induced DNA damage levels and interferes with the fast chromatin relaxation process associated with DNA damage induction.

Conclusions

Advanced fluorescence microscopy techniques enable observation of the effects of oncogene activation in single cells. This study highlights the important role of chromatin organization in the onset of DNA damage and the molecular action of PARP inhibitors.

Laura Maggi **MFAG - 2018**

University of Florence

Effects of adenosine A2A-AR receptor inhibitors on effector functions of T lymphocyte subsets: in vitro tests on human samples and in vivo mouse model

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Background and hypothesis

Adenosine is an endogenous purine nucleoside and its immunosuppressive role in the tumor microenvironment made the Adenosine receptor A2A AR-mediated pathway a possible target for anti-tumoral therapy.

Aims

Develop new synthetic A2A AR antagonists based on a thiazolo[5,4-d]pyrimidine nucleus and characterized by an high affinity for the A2A AR, belonging to two different chemical series, and test their activity on human and mouse T cell functions to a possible further application in cancer immunotherapy

Experimental design

These 5 new developed synthetic A2A AR antagonists supplied by the Chemistry laboratory of the University of Florence, and the known compound ZM 241385, were tested in vitro on proliferation and cytokine production of CD4+ and CD8+ T cells, on the different T helper subsets, and on tumor cell line MCF7, stimulated in presence of Adenosine or its synthetic analogue CGS 21680. Two of the new compounds A2A AR antagonists, and the compound ZM 241385, were tested in vivo on female BALB-c mice, for preliminary toxicity tests.

Results

We found that Adenosine and its agonist CGS 21680-mediated inhibition of proliferation and cytokine production on CD4+ and CD8+ T cells, was restored by ZM 241385 and by some of these new synthetic compounds, even if with different potency. Moreover, these compounds, inhibits in vitro proliferation of tumor cell line MCF7, cultured in presence CGS 21680. Being new compounds never tested in vivo, a preliminary toxicity test with three different doses, was performed using one compound for each series and ZM 241385. Histological analysis of mouse liver and kidney did not show any evident organ damage, nor alterations emerge from ECG and renal perfusion measurements. Flow cytometric evaluation of the main immune cell subsets in the spleen and lymph nodes from mice highlighted minimal percentage variations, whereas no differences were highlighted in the percentage of Treg cells. T lymphocytes functionality, assessed by cytokine production and Ki67 expression, showed significant variations compared to control mice with a prevalence of an inhibitory pathway, although in some cases in the opposite direction of expected

Conclusions

Data from in vitro experiments suggested that these compounds modulate T cell functions interfering with A2AR-Adenosine axis, whereas data from in vivo evaluation demonstrated their safety: all these data are relevant as preliminary step for next use of these compounds on breast cancer mouse model application.

Vittoria Pagliarini **MFAG - 2020**

Università Cattolica del Sacro Cuore, sede di Roma

Splicing dysregulation as potential therapeutic vulnerability for medulloblastoma

Background and hypothesis

MYC-amplified Group 3 MB is the most aggressive medulloblastoma (MB) subgroup that lacks efficacious targeted therapies and displays poor prognosis. Deregulation of RNA splicing has recently emerged as a featuring trait of this pathology that contributes to the aggressive behavior of MB. Mounting evidence indicated that MYC-amplified cancer cells are intricately sensitive to genetic or pharmacological perturbation of splicing and they strongly depend on functional splicing machinery to survive. Thus, it is conceivable that MYC amplification in Group 3 sensitizes MB cancer cells to splicing-targeting treatment.

Aims

Aim 1: Genome-wide effects of MYC on the transcriptome of MB cells
Aim 2: Splicing-targeting approaches as therapeutic tools for MB
Aim 3: Investigation of non-canonical splicing events in MB patients
Aim 4: Development of pre-clinical tools to interfere with canonical and non-canonical splicing dysregulation in MB

Experimental design

In Aim 1 we evaluated the impact of MYC amplification in Group 3 MB cell lines. In Aim 2 we evaluated the efficacy of several splicing inhibitors on proliferation and viability of MB cells. We performed a transcriptome analysis in MB cells treated with the most efficacious splicing inhibitor to investigate the molecular and cellular pathways affected by the drug. Then, we tested its efficacy in vivo by using intracranial orthotopic xenografts mouse model of medulloblastoma. In Aim 3 we searched for non-canonical splicing in order to identify circular RNAs expressed in MB patients and we characterized regulatory elements and the RNA binding protein involved in their biogenesis. We are investigating the functional relevance of identified transcripts for MB oncogenic features. In Aim 4 we designed decoy antisense-oligonucleotides (ASOs) that are specific for inhibiting the activity of single RBPs identified in Aim 1 and 3. We are testing ASOs for their inhibitory effect towards oncogenic features of MB cells and the most promising ones will be also tested in vivo.

Results

We identified a MYC-dependent splicing program underlying the maintenance of the stemness potential of Group 3 MB cells. We identified the splicing inhibitor, THZ531, targeting the activity of two transcriptional cyclin-dependent kinases, CDK12 and CDK13, as a promising new therapeutic approach for MB. Furthermore, we found a hnRNP C-dependent regulatory mechanism to inhibit the general expression of circular RNAs in Group 3 MB cells, maybe ensuring the integrity of the MYC-dependent oncogenic transcriptome. We designed two different ASOs decoy to interfere with the activity of PTBP1, as the main executor of the splicing program regulated by MYC, and of hnRNP C, the negative regulator of the general biogenesis of circular RNAs. Lastly, we identified MB-specific neoantigens generated from the dysregulation

of the canonical and non-canonical splicing that might be exploited in the near future to target Group 3 MB cells.

Conclusions

Although standard-of-care therapy increases long-term survival rates to nearly 70% of patients, the prognosis of the remaining 30% high-risk MB patients with tumor dissemination and recurrent disease remains poor. Thus, identifying more effective and less toxic therapies for MB patients is an urgent and unmet clinical need. Our work elucidated novel, so far unidentified, regulatory mechanisms underlying the aggressive phenotype of Group 3 MB cells, suggesting innovative splicing-based strategies for their inhibition.

Elisabetta Palazzo

Università degli studi di Modena and Reggio Emilia

Neurotrophin network as a novel target for the treatment of cutaneous squamous cell carcinoma

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Background and hypothesis

Cutaneous squamous cell carcinoma (cSCC) is the second most common form of skin cancer. CD271 and Trk neurotrophin receptors (NTR) regulate cutaneous homeostasis, but their roles in cSCC remain incomplete. We previously identified CD271 in Well-Differentiated (WD) cSCCs, whereas TrkA is most prominent in Moderately/Poorly Differentiated (MD-PD) tumors.

Aims

Given the importance of NTRs in epidermal homeostasis and cSCC pathogenesis, this work aimed to study the involvement of NTs and their receptors in the pathomechanisms leading to SCC formation, progression, and metastatic behavior, with the final goal of identifying new therapeutic targets.

Experimental design

Patient-derived spheroids were generated to mimic cSCC and its subpopulations, including stem-like Rapidly Adhering cells (RAD) and more differentiated Not-RAD cells. Molecular profiling and functional studies were performed. NTR-associated metastatic potential of SCC cells was assessed using a zebrafish avatar model. Novel mouse models with conditional and inducible deletion of CD271 in the epidermis (cKO) were developed to evaluate effects during skin development and under inflammatory and carcinogenic stimuli.

Results

NTR receptors play opposing roles in regulating malignant features, cancer stem cell (CSC) behavior, and inflammation in cSCC. CD271 expression correlates with reduced malignancy features, particularly in RAD spheroids, which are characterized by high aggressiveness and stemness, potentially by modulating keratin-related molecular signatures. Furthermore, CD271 protein processing may play a critical role in this process. In contrast, silencing TrkA or pharmacological inhibition led to decreased spheroid viability, especially in stem-like populations, along with reduced mitogenic signaling and lower NF-κB activity, as well as diminished invasiveness in collagen-based 3D matrices. Treatment with Trk/Fc chimeric proteins induced apoptosis, reinforcing the idea that CSC viability in cSCC is neurotrophin-dependent.

Zebrafish avatar models confirmed that RAD cells were more metastatic than NRAD cells. CD271 activation or Trk inhibition significantly reduced metastasis formation and tumor burden while enhancing leukocyte recruitment, indicating a link between neurotrophin signaling modulation and immune engagement. Notably, immune cell depletion in zebrafish increased metastasis, further confirming the relevance of the immune context. These findings were mirrored in vitro using a macrophage–cancer cell 3D model, where CD271 overexpression led to enhanced monocyte infiltration and differentiation into anti-tumor M1-like macrophages, suggesting that CD271 drives pro-inflammatory, anti-tumoral immune activation.

Furthermore, we assessed how CD271 and Trk modulation affect cSCC responses to photodynamic therapy (PDT). Receptor modulation significantly altered spheroid viability post-PDT. These findings highlight the

clinical relevance of neurotrophin receptor targeting, supporting its potential as a therapeutic axis in cSCC. To explore in vivo relevance, we developed constitutive and inducible conditional epidermal CD271 KO mouse models. These mice showed increased susceptibility to skin tumorigenesis in a chemical carcinogenesis protocol, with disorganized epidermis, increased proliferation, and decreased differentiation markers. Transcriptomic analysis of KO skin highlighted deregulation of PI3K/AKT/mTOR, MAPK, and integrin signaling pathways, as well as an increased inflammatory microenvironment.

Conclusions

Our results indicate that NT receptors contribute to maintaining active cancer-related pathways and could be a target for novel therapeutic strategies. Globally, our data strongly designate CD271 as a key regulator of skin homeostasis and suggest its involvement in skin pathological processes.

Nunzia Pastore **MFAG - 2020**

Telethon Institute of Genetics and Medicine (TIGEM)

Tackling the biological complexity of Hepatocellular Carcinoma in Alpha-1 Antitrypsin Deficiency

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Background and hypothesis

Chronic liver diseases are major risk factors for hepatocellular carcinoma (HCC), the second leading cause of cancer-related mortality worldwide. Alpha-1 antitrypsin deficiency (AATD) is a genetic liver disorder characterized by the accumulation of mutant alpha-1 antitrypsin Z (ATZ) within the endoplasmic reticulum (ER) of hepatocytes. This pathological retention causes chronic liver injury, fibrosis, and cirrhosis, and significantly increases the risk of developing HCC. Despite progress in understanding its pathophysiology, liver transplantation remains the only curative option for AATD-related liver disease.

The buildup of ATZ in the ER activates hepatotoxic stress responses and disrupts liver regenerative response, creating a permissive environment for HCC. However, the molecular mechanisms linking ATZ accumulation to tumorigenesis are not fully understood.

We hypothesize that ATZ retention in hepatocytes interferes with progenitor cell differentiation during liver development and/or triggers hepatocyte de-differentiation in adult liver, leading to dysregulated gene expression and impaired tissue homeostasis. These alterations may compromise regenerative responses and contribute to malignant transformation.

Aims

This study aims to:

1. Elucidate the mechanisms driving impaired hepatocyte function in AATD.
2. Identify the cell(s) of origin contributing to HCC development in the context of AATD.

Experimental design

To explore these mechanisms, we employed a combination of in vivo and in-vitro models, including PiZ mice (a validated AATD model), liver organoid culture, and precision-cut liver slices. These models were used to identify molecular pathways associated with altered metabolic and regenerative functions leading to HCC.

Additionally, we integrated lineage-tracing techniques and liver injury/regeneration protocols to induce transient proliferative states, enabling us to track the fate and contribution of hepatic cell populations to tumor development.

Results

Our findings highlight the NOTCH signaling pathway as a central regulator of both neonatal and adult manifestations of AATD liver pathology. NOTCH plays a critical role in biliary differentiation, progenitor cell function, fibrosis, inflammation, and tumorigenesis.

We observed that activation of NOTCH signaling, particularly via its ligand JAG1, is elevated in adult hepatocytes and correlates with disease severity in both PiZ mice and human liver samples. These results suggest that NOTCH signaling may simultaneously modulate liver repair and drive disease progression,

In addition, our data identify hepatocytes and progenitor cells as major contributors to the onset and evolution of HCC in the context of AATD.

Conclusions

By delineating the molecular and cellular landscape underpinning HCC in AATD, particularly the role of NOTCH signaling, this study offers new insights into disease pathogenesis. These findings could inform the development of targeted therapies, potentially offering alternatives to liver transplantation and improving outcomes for AATD patients at risk of liver cancer.

Alberto Puliafito **MFAG - 2020**

Candiolo Cancer Institute, FPO - IRCCS

Deciphering the role of population dynamics and phenotypic plasticity in metastatic colorectal

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Background and hypothesis

Intra-tumoural heterogeneity in metastatic colorectal cancer (CRC) is one of the underlying causes of incomplete therapeutic response to targeted therapies and of the emergence of minimal residual disease. Such a biological state is characterized by slow cell proliferation, phenotypic plasticity and drug tolerance. Remaining tolerant cells are particularly interesting because they can help fueling relapse and ultimately leading to the emergence of drug resistance.

Aims

Understanding tumor phenotypic hierarchies in this context might indeed help identifying particular phenotypic states prone to tolerance and therefore finding complementary therapeutic strategies. We therefore aim at identifying distinct populations within metastatic colorectal tumors, dissect population hierarchies and find actionable molecular players that can influence the population structure in order to optimize the existing therapeutic options.

Experimental design

Here we use patient derived tumor organoids (PDTO) to model population dynamics before and after treatment with Cetuximab (a monoclonal antibody against EGFR). By combining single cell transcriptomics, CRISPR knock-in technology, quantitative live imaging and computational methods we can identify and follow distinct populations within PDTOs, with the aim of deciphering lineage hierarchies.

Results

We show that the population structure in untreated PDTOs can be described by a dual stem (LGR5+) vs differentiated (KRT20+) paradigm, coherently with literature. We identify molecular actuators (i.e. cytokines or inhibitors) that can be leveraged to alter the population structure in favor of either population.

Interestingly, new populations emerge under treatment, one with a distinct secretory progenitor phenotype and one enriched in WNT-related genes. We provide evidence that the latter is formed by cells that continue to cycle even under treatment and that its relative abundance increases after therapy.

In order to dissect the lineage hierarchy among the different phenotypes we develop an inference method suitable to dissect phenotypic plasticity in cell populations.

Conclusions

Our results show a particularly rich phenotypic landscape which unveils complementary pharmacologic vulnerabilities and poses a more general question of how to design a ‘dynamical’ drug treatment.

Concetta Quintarelli **MFAG - 2020**

Ospedale Pediatrico Bambino Gesù

Pushing Boundaries: Next-Gen CAR Cell Innovations for Leukemia Treatment

Background and hypothesis

Chimeric Antigen Receptor (CAR) therapy has emerged as a groundbreaking and highly effective form of immunotherapy for the treatment of leukemia. CAR T cells targeting CD19 have demonstrated impressive clinical efficacy in B-cell malignancies, leading to the approval of multiple commercial therapies. However, significant challenges remain. Notably, 30–60% of patients experience relapse following CD19-directed CAR T-cell infusion, with 10–20% of these relapses attributed to the emergence of CD19-negative leukemic clones—highlighting the urgent need to identify alternative B-cell-specific targets.

Moreover, even patients who relapse with CD19-positive disease often do so due to delays in the manufacture and delivery of personalized, autologous therapies. These delays are particularly detrimental in individuals with severe lymphopenia or rapidly progressing disease following hematopoietic stem cell transplantation. Such limitations underscore the critical need for a readily available, off-the-shelf therapeutic solution.

In this context, allogeneic cell sources present a promising alternative. In this project, we demonstrate that both allogeneic donor-derived T cells and third-party natural killer (NK) cells serve as robust and effective platforms for the generation of CAR-engineered immune cells, offering new avenues for scalable and timely leukemia treatment.

Aims

We proposed to overcome the limitations associated to autologous CAR T cells, by the development of allogenic CAR products for the treatment of patients affected by precursor B-cell lymphoblastic leukemia. This primary aim was achieved towards the following lines of research:

1) Development of allogenic CAR.CD19 NK cell platform
2) Development of allogenic-donor derived CAR.CD19 T cell platform
3) Optimization of the allogenic CAR cell platforms in terms of efficacy and safety

Experimental design

1) Development of allogenic CAR.CD19 NK cell platform

We optimized the GMP large scale manufacturing of an allogenic third-party CAR NK off-the-shelf bank. In particular, the approach is based on the selection of NK cells from the apheresis performed in healthy donors, and ex-vivo expanded with a feeder-free serum-free strategy. The primary NK cells are genetically modified with a retroviral vector carrying CAR.CD19. Pre-clinical in vitro and in vivo data have been generated to confirm that clinical grade CAR.CD19 NK cells preserve their potency and anti-leukemic activity.

2) Development of allogenic-donor derived CAR.CD19 T cell platform

For relapsing patients with significant leukopenia after transplant, we manufactured CAR.CD19 T cells from allogenic donors. The allogenic CAR.CD19 product was first tested in 13 patients under a hospital-exemption setting, and currently is applied in a formal Phase I/II clinical trial.

3) Optimization of the allogenic CAR cell platforms in terms of efficacy and safety

To further optimize the system, we pre-clinically developed: A) a bispecific CAR construct able to simultaneously target CD19 and CD22, with the aim to reduce antigen escape in the treated patients; B) a gene edited CAR product to reduce the inhibitory signaling in NK cells exerted by the NKG2A/HLA-E axes; C) a strategy to overcome systemic toxicity in patients receiving CAR T cells.

Results

Clinical grade CAR NK cells have been developed on a large scale up to the manufacturing of 20 doses from one single donor. The CAR NK products have been deeply characterized for CAR expression, cell composition, viability, vector copy number, endotoxin, mycoplasma and microbiological contamination, as required by Pharmacopeia for ATMPs. IMPD for the evaluation of a clinical trial have been also finalized for submission.

Allogenic CAR.CD19 T cells have been tested in the clinical setting in 13 children/young adults. The toxicity profile was comparable with that of autologous CAR-T cells, characterized mainly by cytopenia, cytokine release syndrome (maximum grade 1), and grade 2 immune-effector cell-associated neurotoxicity syndrome. One case of acute graft-versus-host disease (GVHD) occurred and was rapidly controlled with steroids and ruxolitinib. None of the other patients, including 3 given ALLO-CAR-T cells from an HLA-haploidentical donor, experienced GVHD. Two patients received ALLO-CAR-T cells before HSCT and showed a significant expansion of CAR-T cells without any sign of GVHD. All patients obtained complete remission (CR) with absence of minimal residual disease in the bone marrow.

We finalized the pre-clinical study showing high efficacy of CAR.CD19/CD22 to control not only CD19+/CD22+ leukemia, but also leukemia cells characterized by a negative expression of either CD19 or CD22. We optimized a protocol for the effective genome editing of NK cells, and their further genomic modification to incorporate a CAR construct. The genome editing of NKG2A in CAR NK cells show a great advantage in controlling leukemia cells characterized by a high expression of HLA-E. Lastly, we were able to prove that neutralizing IFNγ improves safety without compromising efficacy of CAR cell therapy in B-cell malignancies.

Conclusions

Allogenic anti-CD19 CAR cells are emerging as a transformative approach in adoptive immunotherapy, with the potential not only to bypass the logistical and manufacturing hurdles of autologous therapies but also to broaden patient access and improve clinical outcomes.

Francesco Raimondi **MFAG - 2020**

Scuola Normale Superiore

Exploring anti-cancer, drug-repurposing opportunities through GPCRs targeting and AI.

Background and hypothesis

G protein coupled receptors (GPCRs) are the largest family of transmembrane receptors and target of one third of approved drugs and they are emerging as key players in the tumor micro-environment. Therefore they represent attractive targets for drug repositioning in cancer.

Aims

I will discuss recent computational strategies that we developed to suggest new anti-cancer therapies via drug repurposing and exploitation of large scale cancer transcriptomic and drug perturbation datasets.

Experimental design

We expanded the GPCR signaling network, both at the intracellular level by using experimental and predicted transducer interactions, as well as at the extracellular level by considering both ligands (peptidic and organic) and biosynthetic enzymes. We then inspected cancer transcriptomics datasets finding out that multiple GPCRs are differentially regulated together with their upstream partners, either ligands or enzymes, across cancer subtypes.

We also leveraged cancer cell lines (CCLs) drug sensitivity datasets to predict drug response using cell line transcriptomics, focusing on models' interpretability and deployment on patients' data. We used large language models (LLMs) to match drug to mechanisms of action (MOA)-related pathways. Genes crucial for prediction are enriched in drug-MOAs, suggesting that our models learnt the molecular determinants of response. Furthermore, by using only LLM-curated, MOA-genes, we enhanced the predictive accuracy of our models. To enhance translatability, we aligned RNAseq data from CCLs, used for training, to those from patient samples, used for inference.

Results

GPCR ligand (or biosynthetic enzyme) concordant expression improved patient stratification based on survival, suggesting a synergistic role for the activation of GPCR networks in modulating cancer phenotypes. We identified many such axes across several cancer molecular subtypes, including many involving receptor-biosynthetic enzymes for neurotransmitters. We found that GPCRs from these actionable axes, including, e.g., muscarinic, adenosine, 5-hydroxytryptamine, are the targets of multiple drugs displaying anti-growth effects in large-scale, cancer cell drug screens, which we also validated.

Moreover, we used our AI approach to profile TCGA samples and showed that patients' best scoring drugs match those prescribed for their cancer type. We generated predictions many potential drug repurposing hits, both considering oncological drugs from GDSC and non-oncological drugs from PRISM, including many GPCR ones. We further predicted and experimentally validated effective drugs for the patients of two highly lethal solid tumors, i.e., pancreatic cancer and glioblastoma. Finally, we have released our general-purpose predictive method, called CellHit, through a a freely available webserver (https://cellhit.bioinfolab.sns.it/).

Conclusions

The combined usage of curated resources for GPCR signaling to interpret cancer genomics dataset, and AI approaches trained large scale cancer cell drug screening, have lead to the identification of potential drug hits for repurposing in specific cancer contexts.

Giovanni Sorrentino **Start-Up - 2020**

International Centre for Genetic Engineering and Biotechnology (ICGEB) Padriciano 99, 34149 Trieste, Italy

An organotypic model of ductular reaction reveals a mevalonate-dependent vulnerability in reactive biliary cells

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Background and hypothesis

Chronic liver diseases such as Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD), cholangitis, and viral hepatitis are marked by persistent damage that triggers the ductular reaction (DR)—a maladaptive reparative response involving the expansion and phenotypic reprogramming of biliary epithelial cells (BECs). DR reshapes the hepatic microenvironment, contributes to fibrosis and fosters a pro-tumorigenic niche, acting as a key driver of liver cancer development. Despite its pathophysiological and clinical relevance, the mechanisms underlying DR activation remain poorly understood, partly due to the lack of physiologically relevant experimental models.

Aims

We aimed to develop a complex ex vivo organotypic model of DR that preserves native tissue architecture, cellular heterogeneity, and both cell–cell and cell–matrix interactions, enabling the investigation of the multifaceted mechanisms underlying DR.

Experimental design

We generated a novel organotypic model of DR using precision-cut liver slices (PCLS) from mouse and human livers, cultured in a matrix-supported expansion medium enriched with niche factors. This system recapitulates ex vivo both the propagation of DR from previously injured tissue and de novo induction in metabolically compromised livers. We integrated single-nucleus RNA sequencing (snRNA-seq), digital histology, and immunofluorescence in PCLS, and used cholangiocyte organoids as a reductionist system to investigate cell-autonomous mechanisms. Finally, we validated findings using patient-derived samples, retrospective clinical data and a cohort of MASLD patients stratified based on statin use.

Results

Mouse and human PCLS retained complex tissue architecture and supported robust expansion of reactive BECs, accompanied by stromal activation, immune retention, and hepatocyte plasticity. Specifically, snRNA-seq identified proliferative BEC populations, active myofibroblasts and metaplastic hepatocytes. A focused metabolic screen identified the mevalonate (MVA) pathway as a critical regulator of DR. Statins, inhibitors of this pathway, suppressed DR ex vivo and in vivo, without impairing hepatocyte proliferation. Mechanistically, MVA-derived cholesterol and geranylgeranyl pyrophosphate (GGPP) cooperatively sustained ERK signalling in reactive BECs. These findings were recapitulated in patient-derived cholangiocyte organoids. Importantly, biopsies from MASLD and PSC patients revealed activation of MVA genes in DR cells. Finally, in a retrospective clinical cohort, statin use correlated with reduced DR and decreased myofibroblast and macrophage accumulation.

Conclusions

This study establishes a physiologically relevant ex vivo platform to investigate the mechanisms and consequences of DR in chronic liver disease. We identify the MVA pathway as a conserved metabolic vulnerability in reactive BECs, essential for their proliferation and linked to pro-fibrogenic and pro-tumorigenic remodelling. These findings suggest that statins, beyond their cardiovascular benefits, could be repurposed to mitigate DR-driven pathology and reduce liver cancer risk in patients with chronic liver injury.

Francesco Spallotta **MFAG - 2019**

Istituto Pasteur Italia - Fondazione Cenci Bolognetti

Metabolic-epigenetic crosstalk harnesses pancreatic cancer onset and progression

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Background and hypothesis

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers with poor prognosis and therapeutic outcome. Several epidemiological studies show that chronic metabolic diseases, such as obesity and type II diabetes, are associated with an increased risk of PDAC incidence and worse clinical outcome. In these conditions, the prolonged exposure to altered metabolism induces aberrant fluctuation of metabolites which can support cancer development, modifying both the epigenome and desmoplastic stroma evolution. Here, we are testing whether: 1) correlation between metabolism and epigenetics associated with PDAC onset might open to novel PDAC vulnerabilities to exploit as therapeutic targets; 2) analyzing how dysmetabolism affects pancreatic cancer associated fibroblast functions in PDAC.

Aims

Our research aims at: 1) identifying an epi-metabolic landscape associated with impairment of DNA methylation related enzymes caused by dysmetabolism in pancreatic tumorigenesis; ii) understanding the function of metabolic alteration on PDAC stroma development; iii) revealing novel PDAC vulnerabilities to be exploited as biomarkers or therapeutic targets.

Experimental design

Dysmetabolism effect on PDAC development was investigated in: 1) LSL-KrasG12D; PDX-1-Cre mice (KC mice) exposed to high-fat diet (HFD); 2) HFD-fed wild type immunocompetent syngeneic mice by orthotopic transplantation of pancreatic intraepithelial neoplasia (PanIN) organoids; 3) human pancreatic duct epithelial cells bearing KRAS mutation (HPDEmut) exposed to dysmetabolic mimicking medium; and 4) primary pancreatic cancer associated fibroblasts (CAFs) isolated from chemotherapy naïve PDAC patients with/without an history of metabolic syndrome.

Results

For what concerns tumorigenesis, untargeted metabolomics on pancreatic samples highlighted free fatty acid level alteration during pancreatic tumorigenesis upon dysmetabolic condition. Moreover, targeted metabolomics, showed higher S-adenosyl methionine (SAM) and succinate (SA) levels and lower levels of α-ketoglutarate (aKG) in HFD mice, prompting to focus on ten-eleven dioxygenase 1 (TET1)/ thymine DNA glycosylase (TDG) DNA demethylation complex. Interestingly, epi-metabolite level alterations were paralleled by TET1/TDG complex dissociation and consequent accumulation of iterative cytosine modifications, including 5-formilcytosine (5fC). These findings were validated in an in vitro model of HPDEmut exposed to a combination of free fatty acid and high glucose, mimicking dysmetabolic condition, revealing TDG malfunctioning upon these culture conditions. Molecular dynamics and mutational analyses showed that SA, increased upon dysmetabolic condition, binds TDG on Arg275 inducing its hyperactivation. Accordingly, αKG administration restored DNA demethylation cycle reducing 5fC and abasic sites levels in HPDEmut

exposed to dysmetabolic conditions. Moreover, for what concerns PDAC progression the dysmetabolic-associated higher PDAC aggressiveness was paralleled by collagen fibril enrichment due to prolyl 4-hydroxylase subunit alpha 1 (P4HA1) increased function. Upon dysmetabolism, P4HA1 boosts collagen proline hydroxylation, intensifies collagen contraction strength, precluding PDAC infiltration. Noteworthy, semaglutide, an incretin agonist, prevents the higher dysmetabolism-dependent PDAC stromal deposition and allows T lymphocyte infiltration, reducing tumor development.

Conclusions

These results shed light on novel therapeutic options for PDAC patients with metabolic syndrome aimed at PDAC stroma reshape and TDG function targeting.

Federica Ungaro **MFAG - 2019**

Università Vita-Salute San Raffaele

The role of MFSD2A in the resolution of colorectal cancer-promoting inflammation: implications for innovative therapies.

Background and hypothesis

The intrinsic connection between inflammation and tumor promotion is well-established in colorectal cancer (CRC), where chronic inflammation, like ulcerative colitis (UC), plays a significant role in intestinal carcinogenesis. UC patients are at increased risk of developing colitis-associated cancer, reinforcing the connection between inflammation and cancer. Previous studies from our laboratory revealed that UC patients with active inflammation have impaired production of inflammation-resolving DHA-derived metabolites due to a defect in the endothelial Major Facilitator Superfamily Domain containing 2A (MFSD2A). Given the MFSD2A's role in reducing colonic inflammation, we hypothesize that it also counteracts colorectal cancer-associated inflammation.

Aims

- 1. To define the functional role of MFSD2A in orchestrating pro-resolving pathways in the intestinal endothelium of CRC patients.
- 2. To generate an MFSD2A-inducible mouse model to assess the functional role of this protein in different phases of experimental CRC development.
- 3. Evaluate the clinical efficacy of cell-specific Mfsd2a overexpression in experimental models of non-metastatic CRC.

Experimental design

Human Intestinal Microvascular Endothelial Cells (HIMEC) isolated from CRC and healthy samples were transduced with lentiviruses carrying either the GFP-tagged MFSD2A encoding sequence (MFSD2A-OE), or the relative GFP control, or the MFSD2A-targeting shRNAs (shMFSD2A), or the scramble as control (shCTRL), then analyzed by transcriptomics and lipidomics. A colon-adenocarcinoma cell line (Caco-2) was cocultured with either MFSD2A-OE or GFP CRC HIMEC to evaluate their proliferation rate. An orthotopic CRC model was performed by intrarectally injecting CD1 nude mice with a cell mixture of Caco-2 and CRC HIMEC overexpressing either MFSD2A or GFP as a control. Collected tumors were analyzed by flow cytometry, single-cell RNAseq, and lipidomics.

Results

CRC HIMEC display a pro-resolving lipidomic profile linked to higher MFSD2A expression compared to healthy cells. However, gene expression analysis shows a pro-inflammatory profile in CRC HIMEC, suggesting that MFSD2A levels alone are insufficient to induce a fully pro-resolving phenotype. We performed loss-of-function experiments to assess whether the pro-resolving profile in CRC HIMEC depends on MFSD2A. MFSD2A silencing reduced pro-resolving functions, confirming its role in balancing pro-inflammatory and pro-resolving signals in CRC regarding lipid production.

Caco-2 co-cultured with MFSD2A-OE CRC HIMEC displayed a decrease in their proliferation rate, indicating that endothelial MFSD2A overexpression reduces tumor cell proliferation. In vivo, mice injected with a Caco-2/MFSD2A-OE CRC HIMEC mixture developed lighter and smaller tumors with increased pro-resolving lipid release and reduced proinflammatory molecules (Fig. 2D). Additionally, single-cell RNAseq analysis showed an augmented monocyte-to-macrophage activation in MFSD2A-treated mice as compared to the control, results further confirmed by flow cytometry showing increased M2 macrophage polarization, indicating a shift toward a pro-resolving immune response upon MFSD2A overexpression.

Conclusions

These data suggested that MFSD2A might contrast the colorectal cancer-associated inflammation, ensuring the correct balance between pro-inflammatory and pro-resolving milieu.

Rossella Venditti **MFAG - 2020**

TIGEM

Dissecting the role of membrane contact sites in cancer progression and drug resistance

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Background and hypothesis

Eukaryotic organelles preserve their identity and function through the continuous flux of lipids and proteins that relies on several mechanisms: cytosolic exchange, vesicular trafficking and, in some cases, local synthesis. The Endoplasmic Reticulum (ER) is a central hub where both synthesis and transport occur. Over the last years, membrane contact sites (MCSs) have been identified as a crucial route for inter-organellar communication. MCSs are sites where different organelles come into close apposition (10-30 nm) without fusing. The ER creates contact (ER contact sites) with almost all the organelles, including the plasma membrane (PM), the Golgi complex, endosomes, and mitochondria.

Many functions occurring at MCSs interface have been discovered, including lipid exchange, signaling events, organelle positioning and membrane fission. Sphingolipid synthesis has been demonstrated to be controlled by MCSs, thanks to the activity of lipid transfer proteins (LTPs), but there is few evidence to support that SLs levels may control the formation/maintenance of MCSs.

Aims

Although the study of ER-Golgi and ER-endosome MCSs has demonstrated their
importance in controlling many crucial cell activities, and although the catalog of MCS components is steadily increasing, a systematic analysis of their composition, regulation and function has not been
performed so far in cancer cells nor has their role in cancer development and progression processes been explored.

The aim of this proposal is to explore and to provide insights into how ER-Golgi and ER-endosome MCSs are regulated during cancer development
and how the functions regulated by these MCSs may affect cancer progression and dissemination mechanisms.

Experimental design

To dissect the role of ER-mediated contact sites in cancer progression we analyzed the regulation of ER-Golgi and ER-late-endosome MCSs using control and breast cancer cell lines, identifying their molecular composition. We have developed a system of organelle reporters that allow us not only to visualize ER-Golgi contact sites by light microscopy but also to perform high-content screening (HCS) in a robust and reproducible manner. We took advantage of a strategy based on fluorescently-tagged transmembrane reporters of the two organelles of interest to monitor the MCSs dynamics.

These tools allow us to identify novel regulators of contact sites. We performed experiments to evaluate the impact of those components in lipid transport and in cell motility.

Results

Among all the functions occurring at MCSs interface, sphingolipid synthesis has been demonstrated to be controlled by MCSs, thanks to the activity of lipid transfer proteins (LTPs), but there is few evidence to support that SLs levels may control the formation/maintenance of MCSs. The ceramide transfer protein CERT transfers ceramide from the ER to the TGN, where ceramide is converted into sphingomyelin. We found that CERT uses the ER-TGN MCS to transfer ceramide and controls their stability. CERT acts as a regulatory protein at the MCSs, as its depletion results in the increase in ER-TGN MCSs, that is reverted by inhibiting Cer synthesis, suggesting that the increase in ER-TGN contacts depends on the ability of CERT to control ceramide flux. A lipidomic analysis revealed that a major change induced by CERT depletion is the increase in glucosylceramide (GlcCer) levels, consequent to a larger fraction of Cer that is trafficked via vesicular transport to the cisGolgi where it is converted into GlcCer. We realized it is GlcCer that is responsible for the increase in ER-TGN contacts, and that it is FAPP2, a GlcCer LTP, that mediates this stabilization effect of GlcCer. A similar role of GlcCer and of FAPP2 is found under conditions that lead to the accumulation of GlcCer, i.e. the defective degradation of GlcCer in GBA1-depleted cells. Our data reveal a novel mechanism tuning the ER-TGN CS, which is mediated by GlcCer and by the GlcCer sensor FAPP2.

In addition to this, in yeast, one protein has been identified to act similarly to CERT ,the Nvj2p, an ER resident protein enriched at the nucleusvacuolar junction, senses the ceramide (Cer) levels and moves to the ER-Golgi MCSs promoting the non-vesicular transport of Cer towards the Golgi. The Nvj2p mammalian homolog is TEX2, an ER protein recently shown to reside at ER-LE/Lys contact sites.

To investigate whether TEX2, similarly to its yeast homologue, may act at multiple ER-mediated contact sites classes, we defined its interactome map. Surprisingly, we found the mitochondrial FUN14 domain containing 1 (FUNDC1) protein as one of the top hits among the identified partners. FUNDC1 is an outer membrane mitochondrial protein that acts at ER-mitochondria MCS during hypoxia-induced stress and controls DRP1 activity to drive mitochondrial fission. We found that TEX2 is enriched at the ER-mitochondria contact sites. We next analyzed the mitochondrial morphology upon TEX2 depletion, and we observed an elongated mitochondrial network. This mitochondrial phenotype, which is reminiscent of that induced by FUNDC1 depletion, was reverted by the over-expression of DRP1, indicating that TEX2-depleted cells need higher DRP1 levels to operate proper mitochondrial fission. These data, together with the observation that Tex2-depleted cells exhibit lower mitochondrial fission rate, impaired ER-mitochondria contacts, and a drop in the number of mitochondrial nodes, support the hypothesis that TEX2, acting in concert with FUNDC1, creates conditions permissive for DRP1-mediated mitochondrial fission at the level of ER-mitochondria CS.

Conclusions

Further studies will determine to what extent these mechanisms play a role in the several pathological conditions characterized by increased GlcCer levels, including cancer dissemination. We are currently addressing these important questions by using mammosphere system and by tuning both levels of the identified hits, and by controlling the sphingolipid compositions.

POSTER PRESENTATIONS

Francesco Boccalatte **Start-Up - 2021**

Candiolo Cancer Institute, FPO-IRCCS

3D chromatin hubs as headquarters of gene regulation in T-cell acute lymphoblastic leukemia

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Background and hypothesis

Over the past decade, thanks to the expansion of dedicated assays and computational approaches, the three-dimensional (3D) structure of chromatin has been recognized as a key mechanism for regulating gene expression and, consequently, cellular functions. Changes in chromatin structure occur at multiple levels, including A/B compartments, topologically associating domains (TADs), and chromatin loops. These structural modifications enable long-range interactions between enhancers and promoters of different gene classes, including oncogenes. We and others have previously demonstrated that chromatin architecture orchestrates specific epigenomic drivers in lymphoblastic leukemia. Here we offer a genome-wide characterization of enhancer-promoter interactions in pediatric T cell lymphoblastic leukemia (T-ALL), defining chromatin loci with high interactivity and multiple connections (chromatin hubs) as key determinants of leukemic cell identity and survival.

Aims

1) to demonstrate that chromatin hubs are determinants of leukemia identity and progression
2) to identify the heterogeneous representation of 3D chromatin hubs in leukemic subpopulations
3) to interfere with key chromatin hubs, demonstrating their essentiality for leukemia survival

Experimental design

We analyzed chromatin conformation, accessibility and transcription in a large cohort of patients affected by T-ALL to generate a comprehensive map of chromatin hubs. Nine selected cases were analyzed using HiChIP with the H3K27 acetylation mark to identify active enhancers and promoters. Our in-house computational pipeline assessed chromatin interactions by strength and number of loops. We correlated highly interactive loci with their corresponding gene expression and tested significant disease-specific anchors using in silico and CRISPR interference screenings. Additionally, we examined single-cell chromatin accessibility (scATAC-seq) to identify different epigenetic clones within a seemingly uniform blast population. Finally, we targeted transcription factors (TFs) at chromatin hubs testing their effect on chromatin architecture and leukemia growth.

Results

We comprehensively profiled primary pediatric T-ALL samples for 3D chromatin conformation, accessibility, and gene expression. Using H3K27ac interactivity, we could cluster healthy and malignant samples and identify high-risk cases. Leukemic blasts showed a higher frequency of chromatin hubs, regulating oncogenes and disease markers. We developed in silico and CRISPR interference screens to identify crucial chromatin topologies for pediatric leukemia growth. This revealed 3D hubs as central to cancer progression, controlled by multiple cis-regulatory regions. We ranked 3D hubs by interactivity and identified gene modules linked to specific cellular states. Single-cell profiling of leukemic blasts showed heterogeneous

epigenetic clones correlating with higher recurrence risk. Finally, we identified transcription factors in disease-specific hubs and found that disrupting MYB dissolved oncogenic hubs and depleted leukemia, offering a novel therapeutic approach.

Conclusions

Our study shows that 3D chromatin hubs coordinate the expression of disease-specific markers and oncogenes in T-ALL. This profiling allows to better characterize and risk-stratify patients, identifying tumor heterogeneity and offering a novel approach to tackle leukemia progression.

Monica Casucci **MFAG - 2022**

IRCCS San Raffaele Scientific Institute

Armoring CAR-T cells against the tumor glycan shield

Background and hypothesis

CAR-T cell therapy proved effective against B-cell tumors and multiple myeloma, but clinical responses have not been as robust against solid malignancies. Aberrant glycosylation is a hallmark of cancers, which usually show an increased branching of N-glycans. We previously reported that surface N-glycans protect malignant cells from CAR-T cells and that inhibiting their synthesis can boost CAR-T cells efficacy by increasing the quality of the immunological synapse and preventing T-cell exhaustion. Recently, we also reported that N-glycosylation blockade can reduce the immunosuppressive capacity of macrophages within the tumor microenvironment (TME).

Aims

The aim of this project is to engineer CAR-T cells to locally express an enzyme able to de-glycosylate tumor and TME cells, thus resulting in improved antitumor activity.

Experimental design

To meet this goal, we are applying a step-by-step process including: i) structural optimization of the deglycosilating enzyme; ii) generation of CAR-T cells expressing the deglycosilating enzyme; iii) functional characterization of armored CAR-T cells in mice reconstituted with a human immune system and engrafted with human tumors.

Results

In collaboration with the Biocrystallography Unit at OSR, we generated multiple variants of the selected enzyme and tried to purify the recombinant proteins. After many failed attempts with human, murine and yeast variants, we succeeded in generating a functional bacterial enzyme. This recombinant enzyme proved able to reduce the expression of surface branched N-glycans products of the GvT-V enzyme in tumor cells and to induce molecular weight shift of the □1 integrin compatible with its de-glycosylation. Tumor cells pretreated with different amounts of this enzyme induced higher transcriptional activation of CAR-expressing Jurkat cells as compared to the untreated counterpart. Similarly, improved lytic activity and activation was observed when challenging primary CAR-T cells against tumor cells pretreated with the enzyme. In light of these results, we started working on the generation of armored CAR-T cells by designing secreted and membrane-tethered versions of the enzyme cloned into a bidirectional LVV carrying the CAR. In parallel, we are dedicating efforts to develop a NFAT-responsive expression platform, which could be relevant to limit systemic toxicity if the secreted version of the enzyme turns out to be the best performing one. While developing the T-cell asset, we also worked to refine our available animal model, i.e., the human hematopoietic stem/progenitor cells-humanized SGM3 mouse model, focusing on the implementation of a human stromal compartment. Importantly, we observed that when stromal cells are rendered glycosylation-defective they completely lose their pro-tumoral and T-cell-suppressive behavior. Moreover, we found that glycosylation blockade in immune and stromal cells injected in mice with unmanipulated tumor cells

improves CAR-T cell efficacy.

Conclusions

We identified and produced an enzyme able to remove N-glycans from the surface of tumor cells thus improving their targeting by CAR-T cells and we are currently working on the generation of CAR-T cells armored with such enzyme. We have also implemented the humanized mouse model with the stromal compartment and proven that de-glycosylation of TME cells is sufficient to increase CAR-T cell performances independently from the glycosylation status of tumor cells.

Alessandro Cicchetti **MFAG - 2022**

Fondazione IRCCS Istituto Nazionale dei Tumori di Milano

Improving the success rate for thoracic radiotherapy through specific cardiac substructure dosimetry: location matters

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Background and hypothesis

Radiation therapy (RT) represents an option for treating lung, lymphoma, oesophagus, and breast cancer. In these districts, the partial inclusion of the heart into the medium-high dose region limits the level of curative RT doses and, thus, the treatment success rate.

The phase 3 trial of RT dose-escalation RTOG 0617 reported the most robust evidence, where the median survival for the enrolled lung patients was significantly worse in the higher dose arm. One contributing factor was the dose to the heart, with volumes receiving > 5 Gy and 30 Gy associated with an increased risk of death.

Our central hypothesis is that it is possible to determine a dose-response relationship for the heart substructures (HSs), leading to the possibility of precise knowledge-based sparing of critical volumes during RT. According to mathematical estimations from the literature, this would lead to a >20% gain in the 2-year Overall Survival (OS2). Such a gain could represent an improvement more significant than those following the introduction of concurrent Chemo-RT or the hypo-fractionated regimens.

Aims

The objectives of this research are threefold.

First, we aim to refine an existing predictive model for 2-year overall survival (OS2) in lung cancer patients by incorporating the impact of radiation-induced damage to cardiac tissues. In particular, we will explore how such damage contributes to survival-limiting toxicity events in a retrospective patient cohort to identify specific dose constraints for the heart substructures (HSs).

Second, in a prospective cohort, we plan to conduct a comprehensive, multi-functional evaluation of Radiation-induced Heart Diseases. This will allow us to identify subclinical parameters, early damage indicators, that reflect how high-dose radiation affects the heart's most sensitive regions.

Finally, we intend to use a mechanistic computational model to analyze and validate the role of these subclinical parameters in altering cardiac function. This approach could give us deeper insights into the biological mechanisms underpinning radiation-related heart injury.

Experimental design

The study is structured into three complementary parts:

Retrospective study: We will develop a model to predict OS2 in stage III patients with locally advanced non-small cell lung cancer (LA-NSCLC). This model will integrate treatment-related variables, such as the prescribed radiation dose, use of chemotherapy, and immunotherapy, as well as dosimetric features of the HSs, including their calcium score. These elements will help us capture both the efficacy of the treatment in controlling the disease and the contribution of radiation-induced cardiac toxicity to survival-limiting events.

Prospective study: In a forward-looking cohort, we will monitor patients over time by measuring cardiac biomarkers and evaluating morphological and functional cardiac parameters at three distinct time points. This longitudinal assessment will enable us to detect early signs of cardiac damage caused by radiation exposure.
In-silico study: Finally, we will conduct numerical simulations to replicate cardiac function using the data collected from clinical tests. This computational modeling will allow us to explore the underlying mechanisms of radiation-induced cardiac dysfunction and support the interpretation of the clinical findings.

Results

Two models have been developed to characterize survival-limiting toxicity (SLT), leveraging both the contribution of radiation dose to cardiac substructures and the estimated dose to circulating lymphocytes. In the first model, the dose to the inferior vena cava (p 0.03 and 22% of reduction in OS2, ranging from14% to 20% when combined with tumor volume) emerged as a significant predictor of OS2 in LA-NSCLC pts, following a propensity score matching analysis based on key clinical variables associated with improved prognosis.
In the second model, a decrease in overall survival was observed with increasing mean dose for circulating lymphocytes, estimated using the EDIC (Equivalent Dose to Immune Cells) approach. In addition, we validated a previously published OS model based on EDIC (calibration slope 0.80 and offset 0.15, likely imputed to introduce immunotherapy). A risk stratification based on median EDIC value showed a percentage of 38 vs 62 in OS2.
These two approaches are intended to be integrated into a comprehensive framework for modeling SLT in the final patient cohort. This integrated model will also incorporate quantitative baseline imaging data, including coronary calcium score, body mass composition, and the presence of pulmonary emphysema. A preliminary version of this model has been computed using an unsupervised cluster analysis, including the dose to the inferior vena cava and the EDIC dose as additional parameters to the standard clinical and treatment factors.
The prospective study is currently ongoing, with patient recruitment still in progress. No interim analyses have been performed to date. All collected data have been entered into a dedicated database, which will serve as input for the in-silico simulation of cardiac functionality using a computational model developed at Politecnico di Milano.
In addition, a robust data pipeline has been established, including (i) automated extraction of dosimetric features and imaging biomarkers, (ii) integration of baseline imaging data (e.g., calcium score, body composition), (iii) the adoption of open-source and commercial tools (HEDOS, HERO Imaging), (iv) the development of a centralized DICOM-based database to enable multicenter collaboration and model validation.

Conclusions

After 30 months of research, Location Matters has made significant progress in understanding and modeling the interplay between cardiac radiation exposure and survival outcomes in patients with locally advanced lung cancer undergoing radiotherapy. Thus far, the results provide a firm foundation for the project's last phase and hold promise for clinically relevant advancements in personalizing thoracic radiotherapy.

Stefano Cinti

University of Naples Federico II

Managing cancer out from lab: liquid biopsy on chip

Background and hypothesis

Breast cancer is the second leading cause of death from cancer in women. Among its various manifestations and therapy responses, triple negative breast cancer (TNBC) comprises 15-20% of BC, and it has an unfavorable prognosis with increased probability of early metastasis, disease recurrence and shorter overall survival. Although 25% of early-diagnosed patients remain disease free for more than 5 years, the poor prognosis of TNBC is associated with a high propensity to distant metastases and shorter survival after recurrence. TNBC is an orphan disease in terms of therapies, and only 1/3 patients responds to chemotherapy. Thus, the identification and evaluation of novel biomarkers and therapeutic agents is a high priority to reduce risks associated to false positives, over treatments and unnecessary follow-up. Recent evidences indicate that groups of microRNA (miRNA), "signature", if validated, could predict tumor relapse and overall survival, thus offering timely treatment alternatives for TNBC. In addition, the adoption of chemometrics and artificial intelligence tools
allows to extract complete information from complex and heterogenous samples, which is the major cause of current biosensors' failure.

Aims

It is aimed to design novel portable cancer diagnostics by combining biosensors, nanomaterial, chemometrics and artificial intelligence, representing a proof-of-concept to detect circulating miRNAs associated to breast cancer. The perspective, in future, to have validated miRNAs predictors, will allow miRNAchip to offer a valuable architecture for monitoring patients status/recurrence and evaluating therapeutic efficacy. The first time combination between biosensors and chemometrics is aimed to create a 2.0 biosensor, leading to a paradigm shift for cancer diagnostics and implementation, the will represent an extendible concept for diverse type of cancers.

Experimental design

There are three connected experimental sections:
1) Design and optimization of highly specific nano-biosensors that undergo different binding mechanisms will signal the presence of various miRNA signature of TNBC as case of study.
2) Production a paper-based multiplexed biosensor for synchronous measurements, with extensively validated models constituting the proof-of-concept for liquid biopsy at point-of-care.
3) Adoption of multivariate analysis to enhance diagnostic specificity and sensitivity in real biologic fluids, mimicking the presence of multiple targets at randomized levels.
Clustering approaches and artificial neural networks will help to overcome the experimental issues which have limited the growth of cancer diagnostics in real world application.

Results

The design of a novel class of cancer diagnostics tool based on multiplexed paper-based biosensor has been

able to detect groups of miRNAs in blood droplet. miRNAchip represents a proof-of-concept platform that, if associated with trials determining validated predictors, is expected giving patients and medical doctors a user- friendly device for timely diagnosis and personalized approaches, increasing odds for survival. As the case of study miRNAs have been selected to evaluate the efficacy of the platforms, for wide application in future.

Conclusions

Sensors system might represent a valuable alternative in cancer management, from diagnosis to treatment management. In particular electrochemical paper-based electrochemical systems offer to be translated to many systems, starting from TNBC as the starting point.

Marco De Giovanni **Start-Up - 2022**

San Raffaele Scientific Institute

Decoding GPR35-Mediated Immune Cell Migration to Solid Tumors

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Background and hypothesis

Effective antitumor immunity critically depends on the coordinated recruitment of leukocytes to the tumor microenvironment (TME). For instance, the presence of cytotoxic T lymphocytes is generally associated with enhanced responsiveness to immunotherapy, whereas the accumulation of immunosuppressive populations—such as myeloid-derived suppressor cells (MDSCs)—often correlates with therapeutic resistance. Notably, while adoptive cell therapies have revolutionized the treatment of hematologic malignancies, their efficacy in solid tumors remains limited, in large part due to insufficient TME homing of engineered T cells. These observations underscore a pressing need to elucidate the molecular mechanisms guiding immune cell trafficking into solid tumors.

Aims

Leukocyte migration is orchestrated by surface receptors that decode directional cues within tissues, among which G-protein coupled receptors (GPCRs) play a pivotal role. We have recently shown that the orphan GPCR GPR35 is selectively upregulated in activated neutrophils and eosinophils, promoting their recruitment during inflammation and infection in response to the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), released by platelets and mast cells. While previous work has implicated GPR35 in supporting tumor progression through its expression on cancer cells and macrophages, whether it modulates immune cell recruitment to solid tumors remains poorly defined. Here, we speculate that GPR35 drives tumor progression by orchestrating suppressve innate immune cell homing to tumors.

Experimental design

To address this gap, we apply a combination of spectral flow cytometry, adoptive transfer assays and advanced imaging approaches

Results

Our Preliminary data suggest that GPR35 expression within immune cells fosters tumor growth by regulating the accumulation of $CD115^{+}$ suppressive monocytes in the TME.

Conclusions

These findings lay the foundation for uncovering novel migratory circuits that shape the immune landscape of solid tumors.

Chiara Di Malta **MFAG - 2022**

Telethon Institute of Genetics and Medicine

Dissecting the role of Glycoprotein-non metastatic-protein B (GPNMB) at the lysosome

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Background and hypothesis

Increased activity of the MiT/TFE transcription factors (TFs), particularly TFEB and TFE3, drives pediatric translocation-renal cell carcinoma (t-RCC) and contributes significantly to kidney cancer in Birt-Hogg-Dubé syndrome. However, the extensive transcriptional network regulated by these TFs complicates direct therapeutic targeting. We hypothesize that identifying specific TFEB/TFE3 target genes that are directly involved in tumor establishment and progression will enable the development of more focused and effective therapeutic strategies.

Aims

To identify and characterize TFEB/TFE3 target genes that contribute to kidney tumorigenesis, with the goal of uncovering novel oncogenic drivers amenable to targeted intervention.

Experimental design

We combined transcriptomic analyses from patient biopsies and tumor cell lines with TFEB ChIP-seq datasets to identify putative oncogenic targets regulated by TFEB/TFE3. Among the top candidates, we focused on GPNMB, a glycoprotein known to localize both at the plasma membrane and within the endo-lysosomal compartment.

Results

GPNMB emerged as the top candidate gene in our integrative analysis. It is a transmembrane protein enriched on the surface of various cancer cell types, where it promotes tumor growth and progression. Although GPNMB also localizes to the endo-lysosomal compartment, its function in this location was previously unknown. Our recent findings indicate that lysosomal localization of GPNMB enhances specific lysosomal functions that, in turn, promote cell migration and invasion, uncovering a novel mechanism by which GPNMB can contribute to the invasive behavior of MiT/TFE-driven tumors.

Conclusions

Our study elucidates the relevance of GPNMB lysosomal localization in MiT/TFE-associated tumors. We provide new insights into the mechanisms underlying tumor invasiveness and identify GPNMB as a promising candidate for targeted intervention in MiT/TFE-driven kidney cancers.

Alessandra Fiore **MFAG - 2022**

University of Verona

Bioenergetic Reprogramming by Mutant p53 Promotes Ferroptosis Resistance in Pancreatic Cancer

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Background and hypothesis

Pancreatic ductal adenocarcinoma (PDAC) accounts for approximately 90% of pancreatic cancer cases and is associated with a dismal prognosis due to late-stage diagnosis, absence of early biomarkers, and pronounced resistance to chemotherapy. TP53 mutations are present in roughly 75% of PDAC cases, leading not only to the loss of wild-type p53 tumor-suppressive functions but also to the acquisition of oncogenic properties that drive tumor progression. We hypothesize that mutant p53 (mut-p53) contributes to this aggressive phenotype not only by promoting metastasis and chemoresistance but also by conferring resistance to ferroptosis—a regulated form of cell death driven by oxidative stress and iron accumulation.

Aims

This study aims to characterize the anti-ferroptotic role of mut-p53 in PDAC cells and to dissect the underlying molecular mechanisms of this resistance. Additionally, we evaluate a combinatorial therapeutic strategy designed to overcome ferroptosis resistance.

Experimental design

To investigate the role of mut-p53 in ferroptosis regulation, we established a series of complementary in vitro assays using CRISPR-Cas9 p53-knockout cells and PDAC cell lines expressing mut-p53. These models were subjected to comprehensive morphological, metabolic, and transcriptional analyses. Furthermore, we developed and patented a novel first-in-class covalent inhibitor targeting PFKFB3, a critical glycolytic regulator, to evaluate its potential in restoring ferroptosis sensitivity.

Results

Our data demonstrate that mut-p53 expression enhances cell viability, reduces ROS and lipid peroxidation levels, and preserves mitochondrial function under ferroptotic stress conditions. Mechanistic analysis revealed that mut-p53-expressing cells undergo a pronounced glycolytic shift upon ferroptosis induction, indicating a reprogramming of energy metabolism as a key driver of ferroptosis resistance. Glycolysis thus emerges as a pivotal anti-ferroptotic mechanism.

To counteract this metabolic adaptation, we tested our covalent PFKFB3 inhibitor and observed that it effectively disrupted the glycolytic shift and restored ferroptosis sensitivity in mut-p53 PDAC cells. Covalent inhibitors offer advantages such as high potency and sustained target inhibition, making this approach particularly suited to overcoming resistance mechanisms in aggressive cancers like PDAC.

Conclusions

Our findings identify mut-p53-driven metabolic reprogramming as a central mechanism of ferroptosis resistance in PDAC. Targeting glycolysis with a covalent PFKFB3 inhibitor represents a promising therapeutic strategy to restore ferroptosis sensitivity and potentially improve treatment outcomes in mut-p53-positive PDAC.

Giorgia Foggetti **Start-Up - 2021**

Vita-Salute San Raffaele University

Determinants of therapeutic sensitivity and drug resistance in EGFR-driven lung adenocarcinoma

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Background and hypothesis

Half of the mechanisms driving resistance to targeted therapy in EGFR mutant lung cancer remain unknown with limited options available to overcome drug resistance and prolong patient survival. By combining ad hoc models of EGFR mutant lung cancer, we can investigate how specific tumor genotypes and phenotypes affect therapeutic sensitivity and induce drug resistance.

Aims

Dissecting the molecular features underlying drug resistance could enable tailoring treatment regimens for patients who no longer benefit from standard therapy.

Experimental design

We leveraged patient-derived models (PDMs), a genetically engineered mouse model (GEMM) of EGFR mutant lung cancer, and in vitro models that recapitulate the human disease to explore mechanisms of limited therapeutic sensitivity or resistance to osimertinib, a 3rd gen. tyrosine kinase inhibitor, TKI, and standard of care for advanced stage disease. In detail, we are investigating the link between KEAP1/NRF2 pathway dysregulation and limited TKI sensitivity in a mutant EGFRL858R and p53floxed GEMM (EPC) and PDMs with engineered KEAP1 inactivation. Moreover, we generated osimertinib-resistant (OR) cell lines from HCC827 (carrying EGFR-E746-A750), that rely on different TKI-resistant mechanisms. We profiled these OR models to study molecular changes compared to baseline conditions and identify potential vulnerabilities of tumors that lost osimertinib sensitivity.

Results

In all OR cell lines that we generated, we observed evidence of epithelial to mesenchymal transition (EMT) that could be associated with drug resistance and tumor aggressiveness. Surprisingly, one out of the three OR cell lines strikingly decreased EGFR protein and mRNA levels. Evidence of loss of EGFR has just been described; however, this event has been associated to a cell state of drug tolerance and the mechanism through which EGFR loss occurs remains to be elucidated. Importantly, in our model EGFR expression could not be recovered upon osimertinib withdrawal excluding the hypothesis of drug tolerance in our setting. Thus, current work focuses on validating our findings to determine whether EGFR loss may be due to an epigenetic mechanism and/or transcriptional repression, or degradation.

We previously demonstrated that KEAP1/NRF2 pathway alterations affect TKI sensitivity. By molecularly profiling mutant EPC tumors, we observed that Keap1 inactivation promotes differential expression of NRF2-related genes in Keap1-deficient Vs Keap1-proficient tumors including genes involved in glutamine/glutathione metabolism. We are confirming these findings in newly-generated GEMM-derived tumor cells and patient-derived organoids engineered for KEAP1 inactivation. These studies will help disentangle KEAP1/NRF2 pathway dysregulation and unveil unique vulnerabilities for this subset of tumors.

Conclusions

In summary, we set up the foundations to investigate molecular mechanisms that modulate drug responses in specific EGFR mutant tumor genotypes. By correlating our findings with clinical data we will identify new biomarkers for EGFR mutant tumors. Indeed, we aim to exploit potential targets in future experiments aiming to improve outcomes and overcome drug resistance towards precision medicine. Overall, this work will contribute to gaining insights into known (EMT and KEAP1/NRF2 pathway dysregulation) and novel (loss of EGFR) mechanisms of resistance to targeted therapy with the ultimate goal to inform treatment intervention at relapse for specific subgroups of patients with lung cancer.

Irene Franco **MFAG - 2021**

Università Vita-Salute San Raffaele

Mutational processes in pre-cancer kidneys

Background and hypothesis

During a lifetime, normal cells accumulate thousands of genetic changes (somatic mutations), including rare cancer driver mutations that critically influence the transition to cancer. In the kidney, the most common cancer subtype originates from epithelial cells of the kidney proximal tubule and is termed clear cell renal cell carcinoma (ccRCC). Proximal tubule cells have high mitochondrial activity compared to the rest of the kidney. Therefore, we hypothesized that the peculiar metabolic organization could be responsible of excessive mutation accumulation.

Aims

We sought to determine what mutational processes are active in human kidney during life and the reasons why the kidney proximal tubule epithelium is particularly prone to transformation.

Experimental design

Cells were isolated from the urine of either control, healthy individuals (n=9), or patients with Von Hippel Lindau (VHL) disease (n=9), a genetic syndrome that predisposes to ccRCC. Somatic mutation analysis in single genomes was achieved by whole genome sequencing of in vitro clonally expanded cells (n=41 VHL+/+ and n=47 VHL+/- clones). Urine-derived clones were also subjected to qPCR measurement of expression of kidney marker genes (231 clones) and RNAseq (n=24 clones) to determine the cell type.

Results

Gene expression analyses showed that our cultured cells were derived from all different nephron segments, including the proximal tubule. Among these cells, we identified a rare cell subset that showed an excess of somatic mutations and a mutational signature (SBS40b) that has previously been identified as a ccRCC-specific mutational signature in large cancer cohorts. Moreover, cells showing SBS40b also displayed an excess of mutations in highly transcribed DNA. These mutations composed a distinct signature that we named ToCCATA (T>C; C>A; T>A on U strand). Normal kidney cells marked by ToCCATA/SBS40b had a unique transcriptional profile, characterized by expression of proximal tubule markers (AQP1, SLC17A3) and kidney injury-repair markers (VCAM1, KIM1, PAX2). The ToCCATA mutational signature was ubiquitously and exclusively observed in ccRCC genomes and in other datasets of injured proximal tubule cells. Importantly, the SBS classes attributed to ToCCATA were enriched among cancer driver SBSs registered in ccRCC, suggesting that the process underlying ToCCATA directly favors the transition to cancer.

Conclusions

Altogether, the unique mutational signature that we have identified in normal and cancer kidney genomes suggests a trajectory of kidney cancer initiation, where ccRCC is not derived from regular proximal tubule cells, but from a cell subset exposed to a specific somatic mutation process. This process is likely linked to an event of kidney injury that suddenly changes cellular metabolism, e.g. environmental hypoxia.

Brenda Green

IFOM

Brillouin Microscopy of breast tumor spheroids On-a-Chip: Mechanical and Transcriptional Responses to Microfluidic-Induced Rapid Deformations

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Background and hypothesis

Mechanical forces and physical properties are essential in regulating cellular processes, with significant implications for both physiological and pathological conditions. In this context, Brillouin Microscopy (BM) is emerging as a breakthrough technology for measuring cellular mechanics in an optical label-free manner. Using custom-designed constricted-based microfluidic devices, we subjected MCF10.DCIS breast tumor spheroids to controlled rapid deformations and monitored their shape recovery in real time with Brillouin and Raman microscopy. This novel platform is used to study how deformations impact nuclear shape, viscoelastic properties, transcriptional changes and invasive potential.

Aims

The aims of this study are to exploit Brillouin and Raman microscopy for providing readouts of mechanical and biochemical properties of breast tumor spheroids on-a-chip subjected to repeated deformations. Finally, the biological effects of these perturbations are investigated.

Experimental design

Microfluidic devices were designed with AutoCAD software and fabricated with soft lithography procedures. Fluid modeling was performed using Comsol software. MCF10.DCIS spheroids were introduced into the device at a constant flow rate and cycled through the deformation channels. Simultaneous detection of Brillouin and Raman light scattering was performed by combining a Raman spectrometer with a multipass tandem Fabry-Pérot interferometer (TFP-2 HC). A green laser with a wavelength of $\lambda = 532$ nm was focused on the sample with a power of 10 mW using 20x long working distance objective (Mitutoyo NA = 0.45).

Results

Brillouin and Raman microscopy on-a-chip is validated using spheroids treated with ROCK inhibitors and a RhoA activator, which perturb actomyosin contractility. Brillouin microscopy showed that ROCK inhibitor treated spheroids were softer than RhoA activator treated spheroids, and this was confirmed by alterations in micro-channel passages times.

Spheroids subjected to sustained compression within micro-channels exhibited a significant increase in Brillouin frequency shifts, which persisted for an extended period. Raman analysis indicated water loss during compression, which we determined was lost from interstitial spaces within the spheroid.

Next, we studied shape recovery dynamics of spheroids inside the microfluidic device. We implemented a modification to the deformation device, which included a spheroid capture zone following the constriction channels. After capturing deformed spheroids, Brillouin and Raman microscopy were conducted to track the spheroids' properties over time. Following 4 cycles of compression, we observed a sustained alteration

in the Brillouin frequency shift, indicating a global stiffening.
Transcriptional changes have been reported for single cells that undergo nuclear deformations and mechanical stress. Motivated by these findings, we examined deformed spheroids using RNA sequencing. We observed a very significant and robust upregulation of the master regulator ATF3 transcription factor after 4 cycles of deformation. Moreover, we observed that spheroids which underwent repeated deformations invaded more in a collagen matrix compared to controls.

Conclusions

Using an innovative platform that combines custom-designed microfluidic devices with Brillouin and Raman spectroscopy, we monitored MCF10.DCIS spheroids deforming in real time, capturing changes in shape, composition, mechanical properties and transcriptional reprogramming.

Federico Gulluni **MFAG - 2022**

University of Torino, Department of Molecular Biotechnology and Health Sciences

A phosphoinositide diffusion barrier prevents aneuploidy and tumorigenesis

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Background and hypothesis

Aneuploidy is a common feature of breast cancer (BC) and is strongly associated with heightened tumor aggressiveness and poor clinical outcomes. Understanding the mechanisms underlying aneuploidy is crucial for uncovering novel therapeutic vulnerabilities. Failures in cytokinesis and resulting tetraploidization are early events that contribute to aneuploidy and tumor progression. Phosphoinositides (PPIns) are known to guide plasma membrane (PM) remodeling during cytokinesis, but their free diffusion poses a challenge for selective localization.

Aims

This study aims to identify mechanisms that restrict PPIn diffusion during cytokinesis and to understand how these mechanisms influence tetraploidization and tumor progression in breast cancer.

Experimental design

The researchers investigated the role of a molecular barrier that limits the lateral diffusion of phospholipids at the intercellular bridge. They focused on the accumulation and function of PI(4)P during early cytokinesis and its interaction with Centralspindlin. Genetic and pharmacological manipulations of PI4KA were used to assess the impact on cytokinesis, ploidy, and tumor progression.

Results

A molecular barrier was identified that restricts movement of PM PPIns and phosphatidylserine at the intercellular bridge, enabling selective accumulation of PI(4)P. This PI(4)P enrichment prevents furrow regression and tetraploidization by facilitating a specific interaction with Centralspindlin at the midbody. Disruption of PI(4)P—via PI4KA knockdown or inhibition—leads to tetraploidization, aneuploidy, and tumor progression. Notably, these changes sensitize tumor cells to low doses of spindle assembly checkpoint inhibitors.

Conclusions

Selective PI(4)P accumulation is crucial for proper cytokinesis and the prevention of tetraploidization in breast cancer. Disruption of this mechanism promotes aneuploidy and tumor progression, but also reveals a therapeutic vulnerability that could be exploited using spindle checkpoint inhibitors.

Nereo Kalebic **MFAG - 2022**

Human Technopole

Role of morphology of glioblastoma stem cells in proliferation and invasiveness

Background and hypothesis

Glioblastoma (GBM) is a major unmet clinical need, showing severe resistance to therapy and a median survival of 15 months. The poor outcome of the therapy is linked to a striking molecular heterogeneity among and within GBM and the infiltrative phenotype, which remains an unresolved biological question. GBM stem cells (GSCs) are thought to be a key cell type underlying the proliferative capacity and invasiveness of GBM.

This study is based on the hypothesis that the morphology of GSC plays an underlying role in GSC proliferation and invasiveness. It builds on my postdoctoral findings that the morphology of neural progenitor cells during brain development plays a key role in their proliferation and cell behaviors. As these cells show a striking resemblance to GSCs, we are translating this concept to tackle the cell biology of GSCs.

Aims

We aim at identifying and characterizing the morphological features of GSCs and linking them with the cell's molecular signature and function. We further aim at identifying morphoregulatory genes and the molecular mechanisms underlying GSC proliferation and invasiveness.

Experimental design

We use patient-derived GBM organoids and cells. Cell morphology is profiled using a series of advanced light imaging methods including time-lapse imaging, whereas molecular fingerprint og glioblastoma stem cells is profiled through various genomics techniques.

We will further perform a high-throughput CRISPR-based perturbation of selected and prioritized morphoregulatory genes in GSCs, which will be followed by a robust readout of invasiveness and proliferation performed in three hierarchical steps, with the easily screenable assays performed first. Most promising genes will be followed up in a 3D co-culture system of GBM and cerebral organoids, which mimics the in vivo scenario of infiltration into healthy tissue. Lastly, an in-depth characterization employing genomics, microscopy, proteomics and xenografting approaches will be performed on the top targets.

Results

We have generated key proof-of-concept data supporting the central hypothesis of the proposal. Building on our preliminary findings, we have demonstrated that the morpho-regulatory protein ADD3 contributes to glioblastoma (GBM) growth and chemoresistance through the formation of cellular protrusions. These results were recently published (Barelli et al., Life Sci Alliance, 2025). Next, we established patient-derived GBM stem cell lines of different morphologies and linked their morphological features with stemness. Finally, we successfully established GBM organoids and obtained transcriptomic profiles of different GSC morphotypes using a custom spatial transcriptomic approach. We have further linked those morphotypes with specific clinically-relevant functions (Barelli et al., Biorxiv, 2025).

Conclusions

Our results so far demonstrate a strong link of glioblastoma stem cell morphology with cancer progression and clinically-relevant features. Identification of GSC morphoregulatory genes as potential drug targets will pave the road to future drug screens and new morphology-informed therapies.

Federica Laudisi **MFAG - 2022**

UNIVERSITA' DEGLI STUDI DI ROMA TOR VERGATA

The antineoplastic role of rafoxanide in colorectal cancer

Background and hypothesis

Drug repositioning is a promising strategy in oncology, and certain anthelmintics, including rafoxanide (RFX), have demonstrated significant anti-tumor activities in colorectal cancer (CRC). We observed that RFX induces endoplasmic reticulum (ER) stress and selectively affects CRC cells, leading to immunogenic cell death and reduced tumor growth, suggesting its potential repurposing as an anti-cancer agent for CRC patients.

Aims

This project aims to characterize the mechanisms underlying the antineoplastic effects of RFX in CRC. In particular, it seeks to determine whether RFX can modulate the CRC microenvironment, metabolism, and gut microbiota, while also assessing its efficacy in limiting colon cancerogenesis at advanced stages of the disease and the spread of metastasis.

The project's initial phase aims to clarify the potential effects of RFX on the CRC microenvironment and whether it influences CRC metabolism.

Experimental design

In the CRC niche, the transcription factors STAT3 and NF-κB are hyperactivated in both malignant cells and TILs and cooperate to maintain cancer cell proliferation/survival and drive protumor inflammation. Thus, we evaluated cell proliferation and/or STAT3/NF-κB activation in RFX-treated colon tissues collected from experimental murine models of CRC, human CRC cells, CRC patient-derived explants and organoids. The STAT3/NF-κB activation and cytokine production were assessed in TILs isolated from CRC specimens and treated with RFX. We investigated the effects of TIL-derived supernatants cultured with or without RFX on CRC cell proliferation and STAT3/NF-κB activation.

The impact of RFX on CRC metabolism was assessed in human CRC cells treated with RFX. The oxygen consumption rate (OCR) and the mitochondrial membrane potential (MMP) were assessed using the seahorse XF24 assay and flow cytometry. Transcriptomic, proteomic and metabolic analysis and quantification of cytochrome C in the cytosolic fractions were performed in RFX-treated CRC cells. Oligomerization of the voltage-dependent anion channel (VDAC)-1 was assessed by Western blotting. ROS levels were quantified by fluorescent probe (DCFDA). The impact of RFX on mitochondrial activity in vivo was explored in a murine model of sporadic CRC.

Results

We observed that RFX negatively affected STAT3/NF-κB oncogenic activity in the CRC microenvironment. In particular, it restrained STAT3/NF-κB activation and colon tumorigenesis in vivo and reduced STAT3/NF-κB activation in cultured CRC cells, CRC-derived explants/organoids, and TILs. Finally, RFX treatment impaired the ability of TILs to produce protumor cytokines and promoted CRC cell proliferation.

Additionally, RFX-treated CRC cells showed significantly reduced basal OCR, ATP production, and maximal

respiration, indicating cellular quiescence. Transcriptomic, proteomic, and metabolic analyses revealed a critical impairment in mitochondrial activity following prolonged RFX treatment and increased cytochrome C release into the cytosol. RFX rapidly enhanced ROS production, blocking complex I and III activity, promoted the open configuration of VDAC1—a key modulator of mitochondrial-mediated apoptosis—and decreased MMP in CRC cells. Treatment with the ROS scavenger N-acetylcysteine limited RFX-dependent VDAC1 opening and mitochondrial membrane depolarization. Proteomic analysis of tumor colonic samples isolated from RFX-treated mice with sporadic CRC demonstrated significant mitochondrial dysfunction.

Conclusions

RFX is a potent inhibitor of STAT3 and NF-κB activation in the CRC microenvironment and negatively impacts CRC metabolism by impairing mitochondrial activity.

Alessandra Lugo **MFAG - 2021**

Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Cancer incidence and mortality attributable to cigarette smoking and exposure to secondhand smoke

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Background and hypothesis

Cigarette smoking remains the leading preventable cause of mortality and morbidity worldwide. Tobacco use is responsible for an estimated 8 million deaths annually, including approximately 1 million deaths caused by exposure to secondhand smoke (SHS). Globally, more than one out of three cancer deaths could be avoided by implementing existing evidence-based prevention strategies and by addressing key modifiable risk factors, including tobacco use. However, significant challenges persist in accurately quantifying the mortality and morbidity attributable to smoking. These difficulties arise in part from the lack of comprehensive assessments that go beyond smoking status to include factors such as smoking intensity, duration, and time since quitting. Similarly, evaluating the health risks associated with SHS exposure remains complex and inadequately addressed in many studies.

Aims

The main aim of this epidemiological project is to provide a comprehensive picture of the burden of cancer attributable to cigarette smoking. Specific aims of the project include: i) to provide updated and accurate quantification of the association between cigarette smoking and SHS exposure and the risk of tobacco-related neoplasms; ii) to quantify the number of deaths and disability adjusted life years that could be prevented by eliminating cigarette smoking and/or SHS exposure; and iii) to develop a dedicated website for the visualization, interaction, and periodic updating of estimates on the burden of smoking-related cancers in Italy.

Experimental design

This project involves the conduction of a series of systematic reviews to provide updated and comprehensive quantification on the association between smoking and SHS exposure and the risk of cancer at any site, based on epidemiological evidence from case-control and cohort studies. The systematic reviews were conducted using an approach that takes into account both an umbrella review and traditional reviews for the identification of original articles published in the scientific literature. The umbrella review (i.e., systematic review of systematic reviews, meta-analyses and pooled analyses) has been performed in October 2022 and then updated in March 2025, identifying a total of 382 reviews. Site-specific meta-analyses on active smoking were performed for cancers of the nasopharynx, oral cavity and pharynx, oesophagus larynx, bladder, and leukaemia. In addition, the association between SHS exposure in non-smokers and the risk of lung, breast, cervical and bladder cancer was investigated. Pooled relative risk of cancer according to smoking status, and dose-response relationships with smoking intensity, duration, and time since quitting were estimated using meta-analytic approaches.

Results

Compared with never smokers, pooled RRs for current smokers were 1.61 (95% confidence interval, CI: 1.40-

1.86) for nasopharyngeal cancer (n=40 original articles on the topic), 3.58 (95% CI: 3.03-4.24) for oral and pharyngeal cancer (n=110), 2.65 (95% CI: 2.35-2.98) for oesophageal cancer (n=175), 1.35 (95% CI: 1.14-1.60) for acute myeloid leukaemia, 0.92 (95% CI: 0.72-1.18) for chronic myeloid leukaemia, 0.61 (95% CI: 0.39-0.96) for acute lymphoid leukaemia, and 0.98 (95% CI: 0.80-1.20) for chronic lymphoid leukaemia (n=51). The relationship with smoking intensity (and duration) was often nonlinear, with cancer risk increasing sharply even at small amounts of cigarettes smoked per day. Meta-analyses on SHS exposure showed an increased risk of lung (24%), female breast (24%), and cervical cancer (52%) in non-smokers exposed to SHS, compared with those not exposed to SHS. In addition, there was a non-significant increased risk of bladder cancer (16%) among non-smokers exposed to SHS. The risks also increased with increasing duration and intensity of SHS exposure.

Conclusions

These meta-analyses reinforce the knowledge on the association between both active cigarette smoking and SHS exposure and the risk of cancer. The observed nonlinear dose-response relationships suggest that even low levels of cigarette consumption can substantially increase cancer risk. These findings emphasize the urgent need for comprehensive tobacco control policies and reaffirm that complete smoking avoidance is the most effective strategy for cancer prevention. These estimates are essential to provide precise and updated quantification of cancer morbidity and mortality attributable to smoking and might be used to call for strict tobacco control policies and reinforce the importance of complete smoking avoidance as the most effective strategy for cancer prevention.

Emilia Maria Cristina Mazza **MFAG - 2021**

Humanitas Clinical and Research Center

DNA Metabolism Controls Immunosuppressive T Cell Function In Cancer Immunotherapy

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Background and hypothesis

Solid tumors are infiltrated by cytotoxic immune cells, but their antitumor activity is counteracted by immunosuppressive CD4+ regulatory T cells (Tregs). Among these, CCR8+ Tregs have been shown to possess the strongest immunosuppressive potential. We observed that CCR8+ Tregs within the tumor microenvironment exhibit significantly fewer DNA double strand breaks (DSBs) compared to CCR8− Tregs and those circulating in the blood. This suggested to us that CCR8+ Tregs may benefit from enhanced genomic stability, allowing them to survive and function more effectively in the hostile tumor environment. Based on this observation, we hypothesized that DNA metabolism and nucleotide sanitation play a central role in preserving genome integrity and sustaining the immunosuppressive function of CCR8+ Tregs in cancer.

Aims

Our primary aim was to define the molecular mechanisms that protect CCR8+ Tregs from DNA damage within the tumor microenvironment. Specifically, we sought to determine whether enhanced DNA metabolism and nucleotide sanitation are responsible for this genomic stability, and how interfering with these processes might affect Treg function, survival, and tumor immune evasion. Ultimately, we aimed to explore whether targeting these pathways could be used to weaken Treg-mediated suppression and improve the efficacy of cancer immunotherapy.

Experimental design

To investigate the mechanisms behind the enhanced genome integrity observed in CCR8+ regulatory T cells (Tregs) infiltrating solid tumors, we first generated a single-cell transcriptomic atlas using samples from a variety of human tumors. This allowed us to identify a distinct upregulation of genes and pathways involved in DNA metabolism and nucleotide sanitation specifically in CCR8+ Tregs. To functionally assess the role of these pathways, we employed both small molecule inhibitors and CRISPR/Cas9-mediated gene knockout in primary human Tregs. We also tested the effects of ectopic overexpression of key nucleotide sanitation enzymes on Treg differentiation from uncommitted precursors and their resistance to toxicity induced by modified nucleosides. Finally, we evaluated how disrupting DNA metabolic pathways affected Treg suppressive function in preclinical tumor models, both as a standalone approach and in combination with anti-PD1 immunotherapy.

Results

We found that tumor-infiltrating CCR8+ Tregs harbored significantly fewer DNA double strand breaks (DSBs) compared to CCR8− Tregs and circulating Tregs, indicating enhanced genome stability. Our transcriptomic analysis confirmed that CCR8+ Tregs overexpress genes related to DNA maintenance and nucleotide sanitation. When we interfered with these pathways, we observed a substantial accumulation of aberrant

nucleotides in the DNA, transcriptional dysregulation of metabolic genes, impaired proliferation and activation, increased DSB formation, and ultimately, cell death. Conversely, overexpressing sanitation enzymes promoted the differentiation of Tregs from naïve precursors and specifically protected them from the cytotoxic effects of modified nucleosides. In preclinical tumor models, we demonstrated that disrupting DNA metabolism reduced the suppressive capacity of Tregs and significantly enhanced the efficacy of anti-PD1 therapy, highlighting a synergistic therapeutic opportunity.

Conclusions

Our findings demonstrate that genome integrity in CCR8+ tumor-infiltrating Tregs is tightly regulated by DNA metabolic pathways and nucleotide sanitation mechanisms. These pathways are essential for preventing DNA damage, supporting Treg survival, and maintaining their suppressive function. By disrupting these processes, we were able to induce lethal DNA damage, block Treg activation and proliferation, and reduce their immunosuppressive capacity. Importantly, this disruption also enhanced the response to anti-PD1 therapy in preclinical tumor models. Collectively, our work reveals that DNA metabolism is a previously unrecognized but critical regulator of Treg function in cancer, and suggests a novel therapeutic strategy to overcome tumor immune resistance.

Rosanna Mezzapelle **MFAG - 2022**

Università Vita Salute San Raffaele

Colony stimulating factor-1 receptor (CSF-1R) inhibition reprograms tumor associated macrophages and impairs mesothelioma growth.

Background and hypothesis

Malignant mesothelioma (MM) is an aggressive tumor associated to asbestos exposure. MM tumor microenvironment (TME) comprises around 30% of infiltrating macrophages (MOs).

High Mobility Group Box 1 (HMGB1) is a chromatin protein crucial in MM onset and development, as it contributes to MOs recruitment and sustains chronic inflammation, promoting a proinflammatory TME. BoxA, an 89-amino acid fragment corresponding to the first domain of HMGB1, antagonizes HMGB1 activity and shows therapeutic effects in MM. BoxA binds to the CXCR4 receptor and induces CD47 internalization, leading to the phagocytosis of tumor cells by MOs. This mechanism of cancer-immune surveillance, termed immunogenic surrender (IGS), identifies MOs as fundamental cells in immune responses mediated by BoxA.

Aims

We aim to understand how macrophages support MM progression and whether their phenotype affects the cell crosstalk in the TME.

To this aim we will investigate the role of the colony-stimulating factor 1 receptor (CSF-1R) pathway which is known to modulate MO proliferation and polarization.

Experimental design

We generated 3D spheroids of MM cells co-cultured with mouse bone marrow-derived macrophages (BMDMs) or human monocytes. To assess the effects of MOs polarization states, we compared spheroid growth in co-cultures with M0 (unstimulated), M1-like, or M2-like polarized MOs. Spheroid growth was measured via imaging, and MO polarization was assessed by qPCR and flow cytometry. To validate MOs contribution to MM progression in vivo, we blocked CSF-1R using a monoclonal antibody in a syngeneic MM mouse model, applying two experimental settings: before and after tumor engraftment. TME composition was analyzed by IHC, flow cytometry, and RT-PCR.

Results

BMDMs initially polarized to M0 acquired a pro-tumoral M2-like phenotype when co-cultured with 3D MM spheroids. M2-like MOs promoted spheroid growth. Treatment with anti–CSF-1R monoclonal antibody (aCSF-1R) shifted MO polarization from an M2- to an M1-like phenotype in co-culture settings. Moreover, conditioned medium from MM cells polarized MOs towards M2-like phenotype, an effect that was reversed upon aCSF-1R treatment. CSF-1R blocking in a syngeneic mouse model of MM delayed tumor growth and extended mice survival. Analysis of TME revealed a reduction in the total number of MOs and an increase in M1-like MOs. Interestingly, treatment with aCSF-1R also influenced HMGB1 localization in tumor cells, increasing its cytosolic levels relative to nuclear levels; this suggests that TAMs may induce tumor cells to secrete more HMGB1 in an attempt to sustain their own growth.

Conclusions

MOs sustain MM growth and aCSF-1R treatment reduces it by skewing TAM polarization toward an M1-like state. This may synergize with BoxA, making MM and other inflammation-driven tumors more responsive to immunotherapy.

Francesca Nazio **MFAG - 2022**

Università di Roma Tor Vergata

EXPLOITING CALCOCO2-DRIVEN MITOCHONDRIA-NUCLEUS INTERPLAY TO OVERCOME MEDULLOBLASTOMA THERAPY RESISTANCE

Background and hypothesis

Medulloblastoma is a malignant developmental tumor that arises in the cerebellum, typically affecting children, and originates from immature neural cells. Recent progresses in genomics, single-cell sequencing, and novel tumor models have updated the classification and stratification of MB into 4 subgroups (Wingless, Sonic Hedgehog, Group3 and Group4), highlighting its complex intratumoral cellular diversity. The current standard of care includes neurosurgical resection, craniospinal irradiation, and cisplatin-based chemotherapy. To date, the role of mitophagy and whether mitochondria dysfunction could contribute to MB onset, aggressiveness and radiotherapy resistance is completely unknown.

Aims

Primed by the proven correlation that autophagy activation in MBSCs supports stemness and metastasization,

and encouraged by our striking preliminary results we will develop an articulated multidisciplinary project plan

to investigate:

1) the complex, context-dependent contributions of mitophagy in MB Group3 aggressiveness and radiotherapy resistance in MB;

2) the pro-oncogenic role of the mitophagy receptor CALCOCO2 (NDP52) in MB Group3;

Experimental design

In order to explore the relevance of mitophagy players in MB stem cells, we will focus on the still underappreciated mitophagy receptor CALCOCO2. We will investigate its role in MB by using in vitro (2D and 3D cultures), ex vivo (MB patients) and in vivo models (orthotopic mouse models).

Results

Here we identified a novel pro-oncogenic role for a mitophagy receptor CALCOCO2 as a regulator of MB cancer stem cells malignant phenotype. In details, we discovered

1) CALCOCO2 as a novel subgroup-specific oncogene in MB G3, whose upregulation acts as an initiating event in the development of MB G3;

2) a dual cytoplasmic-nuclear role for CALCOCO2 as crucial for regulating MB resilience to radiotherapy, highlighting its potential as a critical mediator in the cellular mechanisms that govern radioresistance;

Conclusions

By unraveling the mechanisms underlying CALCOCO2's involvement in treatment resistance and metastasis, our research offers the opportunity to identify novel therapeutic targets and to develop personalized treatment to target specific vulnerabilities in MB G3 tumors.

Alessandro Porchetta

University of Rome Tor Vergata

CRISPR-based Platform for Monitoring DNA Repair Activity and Screening Inhibitors

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Background and hypothesis

Cancer cells often exhibit impaired DNA repair capacity and increased genetic instability. Moreover, the effectiveness of chemotherapy is strongly influenced by the ability of cells to repair DNA damage. Despite significant advances in our understanding of DNA repair mechanisms, the development of tools to study DNA repair enzymes has not kept pace. While standard cell-based assays for monitoring repair activity are well-established, they are typically indirect, labor-intensive, and have limited utility in clinical settings. The development of activity-based sensing platforms for monitoring DNA repair enzymes, along with their integration into clinical settings, holds great promise for advancing early cancer diagnosis and enabling personalized therapies tailored to individual DNA repair profiles. CRISPR-based technologies offer a particularly compelling approach, as they can be readily adapted to generate readouts compatible with point-of-care diagnostics. Moreover, CRISPR has demonstrated robust performance with biological samples and clinical specimens, and shows strong potential for applications in high-throughput screening (HTS).

Aims

The overarching goal of my research program is to develop a synthetic biology toolkit for real-time, activity-based monitoring of DNA glycosylases involved in base excision repair (BER), with a particular focus on the detection of lesions containing 8-oxoG. Central to this effort is the creation of a CRISPR-based platform for real-time sensing of hOGG1 and MutY homolog DNA glycosylase (MUTYH) activity. This platform will also be engineered to support throughput screening (CRISPR-HTS) of small molecule modulators of repair activity.

Experimental design

I have engineered highly specific DNA reaction networks (DNRs) capable of transducing glycosylase activity into downstream CRISPR-powered, ultrasensitive detection signals. First, I had engineered a range of synthetic, damaged nucleic acid substrates to serve as targets for DNA glycosylases. Second, the resulting repair products has been integrated into DNA circuits designed to convert enzymatic activity into CRISPR activators. Finally, I had developed activity-based CRISPR platforms to monitor DNA repair activity in tumor cell lysates. The platform has been also adapted for throughput screening (HTS) of established drug candidates confirming the applicability of the approach. Next steps involves platform validation in cytosolic extracts from commercial wild-type and MUTYH knockout (−/−) HeLa cells. Additional testing will be performed using mouse embryonic fibroblasts from MUTYH−/− mice and lymphoblastoid cell lines derived from MAP patients carrying biallelic MUTYH mutations.

Results

To date, I have developed a ultrasensitive, single-step and real-time CRISPR-based platforms for the activity-based monitoring of hOGG1 in cancer cell lysate (HEK293T, LOD = 14.2 ng/ml; linear detection range from 3 to 300 ng/mL). The platform needs to be validated in clinical specimens of cancer-affected patients. I also

demonstrated that the platform can be used to screen inhibitors of hOGG1 in less than 20 minutes with high selectivity and accuracy. A second, activity-based sensors for MUTYH monitoring is currently object of study, with preliminary data showing the capacity to detect MUTYH activity in a single-step assay format. The platform will be used in vitro to identify modulator of MUTYH activity.

Conclusions

The developed CRISPR-based analytical tools for the real-time monitoring of enzymes involed in BER (hOGG1, MUTYH) represent a new paradigm for testing therapeutic hypothesis concerning DNA repair enzymes, and in the future will be also used to fully explore the implications of enzyme variants in cancer; the adaptation of this tools for HTS of drug candidates can be of impact for cancer, as CRISPR technology shows superior sensitivity and specificity for nucleic acid detection compared to standard affinity binding assays thus facilitating drug discovery.

Linda Quatrini **MFAG - 2022**

Ospedale Pediatrico Bambino Gesù

Glucocorticoid therapy in hematopoietic stem cell transplantation: effect on immune reconstitution and function in cancer patients

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Background and hypothesis

Hematopoietic stem cell transplantation (HSCT) represents the only curative option for many haematological malignancies and an efficient immune reconstitution is necessary for a positive outcome. Since HSCT remains a high-risk procedure associated with many complications, including graft-versus-host disease, a percentage of HSCT recipients needs to receive anti-inflammatory glucocorticoid (GC) therapy. However, GCs have been found to regulate innate lymphoid cells function and to inhibit their differentiation from HSCs. It is known that GCs directly regulate gene expression and chromatin accessibility through their nuclear receptor.

Aims

Our aim is to identify the transcriptional and long-term epigenetic modifications that GC therapy generates on HSCs and understand how they influence their differentiation towards the different immune cell subsets, hampering the correct immune reconstitution following HSCT.

Experimental design

Through RNA- and Assay for Transposase-Accessible Chromatin (ATAC)-sequencing experiments, we characterized the transcriptional signature and the chromatin landscape induced by GC treatment on in vitro cultured HSCs. Furthermore, we evaluated the impact of GC treatment on immune function against leukemia relapse following HSCT in an in vivo model of immunodeficient mice receiving human HSCs and infused with leukemic NALM cells.

Results

We found that short GC treatment increases chromatin accessibility on HSCs. As a result, GC restrict cell cycle progression and favor myeloid-biased differentiation in the short term. Moreover, GCs modify chromatin accessibility in regions associated with genes involved in immune response to stimuli. As a consequence, GCs reduce HSC-derived immune cells anti-tumor activity in the long term.

Conclusions

These data shed light on the molecular mechanism by which GC therapy affects immune reconstitution and function and represent an important step towards the development of novel strategies to improve HSCT outcome in oncologic patients.

Silvia Ravera

Univerisity of Genoa

Mitochondrial dysfunction and excessive fission contribute to a premature aging phenotype in childhood cancer survivors

Background and hypothesis

Although survival rates of childhood cancer patients have improved over the past four decades, childhood cancer survivors (CCS) remain at risk for long-term clinical complications related to chemotherapy and radiotherapy, consistent with premature aging. However, the cellular and molecular bases of these symptoms and signs are still unclear. Among the alterations involved in the aging process, mitochondrial metabolism and the resulting increase in oxidative stress play a pivotal role.

Aims

Thus, this study aims to investigate mitochondrial functional alterations in mononuclear cells (MNCs) from CCS, focusing on oxidative phosphorylation (OxPhos) function, cellular energy status, mitochondrial dynamics, and mitophagy.

Experimental design

Analyses were conducted on MNCs isolated from the peripheral blood of CCS (n = 96), age-matched healthy donors (n = 67), and elderly subjects (n = 80). Luminometric, oximetric, and spectrophotometric analyses were performed to evaluate oxygen consumption rate, aerobic ATP synthesis, and intracellular levels of ATP and AMP. Western blot analyses were used to assess the expression of proteins involved in mitochondrial dynamics and mitophagy.

Results

Data show that OxPhos function and efficiency in CCS were decreased compared to age-matched healthy donors and resembled the profile observed in elderly subjects, leading to a reduced ATP/AMP ratio. Western blot analyses revealed that, although the expression levels of key fusion and fission proteins were unchanged in CCS MNCs, DRP1 phosphorylation was altered, promoting excessive fission driven by MTFP1 and mTOR signaling. Moreover, mitophagy markers were reduced, indicating impaired mitochondrial clearance, along with decreased expression of mitochondrial biogenesis regulators. These findings suggest that mitochondrial dysfunction in CCS MNCs results from impaired energy efficiency, excessive fission, and the accumulation of damaged mitochondria.

Conclusions

In conclusion, the results seem to support a mitochondria-driven contribution to the premature aging phenotype observed in CCS and may provide new insights into the cellular mechanisms underlying long-term complications in childhood cancer survivors.

Francesca Ricci **MFAG - 2022**

Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Blocking therapy resistance before it starts: targeting metabolic vulnerabilities and drug resistance evolution in ovarian cancer

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Background and hypothesis

Ovarian cancer is the deadliest gynaecological malignancy in Western countries, with a 5-year survival rate of approximately 40%. Although 85% of patients initially respond to cisplatin-based therapy, around 70% relapse with a resistant disease. Among the various mechanisms of resistance, metabolic plasticity is emerging as a key factor and potential therapeutic target. The scenario is further complicated by intra-tumor heterogeneity, which drives tumor progression and limits treatments efficacy. Our previous studies demonstrated that the addition of metformin (an antidiabetic drug, showing antitumor activities) to cisplatin delay the onset of resistance in ovarian cancer patient-derived xenografts (PDXs).

Building on existing literature and our preliminary data, we hypothesize that uncovering the mechanisms behind this delay, alongside characterization of metabolic reprogramming and tumor heterogeneity shaped by tumor–microenvironment interactions, will lead to the foundation for more effective strategies to prevent resistance development.

Aims

This project aims to prevent the development of cisplatin resistance through metabolic interventions and to track the evolution of tumor heterogeneity during treatment. We use both human and murine ovarian cancer models, in vitro and in vivo.

Experimental design

Murine ovarian cancer ID8-F3 cells sensitive to cisplatin (S) were used to generate resistant (R) cells by gradually increasing cisplatin concentrations. In parallel, cells were treated with a combination of cisplatin and metformin, referred to as SDM, which led to partial resistance. Resistance was assessed through cytotoxicity assays. Metabolic profiling was carried out by metabolomics, and using a Seahorse® extracellular flux analyser on both S ID8 cells and PDXs, as well as their R and SDM sublines derived from in vitro and in vivo multiple treatments. To investigate mechanisms underlying the delayed onset of resistance, protein and gene expression analyses were performed. Tumor heterogeneity evolution under cisplatin treatment was further explored using single-cell RNA seq technologies (10X Genomics) in a PDX (MNHOC124), which closely reflects the clinical progression of platinum resistance.

Results

Resistant ID8 cells showed a five-fold increase in IC50 compared to parental S ID8 F3 cells, while SDM cells exhibited intermediate sensitivity, with only a two-fold increase in IC50. Metabolic profiling revealed that R cells relied more on oxidative phosphorylation, while S and SDM cells depended more on glycolysis. Similar results were observed in ex-vivo cultures derived from S, R and SDM PDX models. Western Blot analyses indicated that the preventing effect of the addition of metformin is AMPK-dependent and linked to autophagy. The scRNAseq analysis enabled the identification of distinct tumor and microenvironment cell

clusters, whose transcriptional signatures are still under investigation, and revealed potential markers and pathways involved in tumor progression and therapeutic response.

Conclusions

These findings suggest that combining cisplatin and metformin effectively delays the development of cisplatin resistance by modulating tumor metabolic plasticity. Moreover, the scRNAseq provided new insights on the role of tumor heterogeneity in the development of cisplatin resistance in ovarian cancer. Ongoing and future researches will focus on elucidating the underlying mechanisms and exploring more targeted approaches to prevent the emergence of resistance.

Patrizia Romani

Università di Padova

A KEAP1-dependent trade-off between migration and ferroptosis limits RAC1-induced hematogenous dissemination

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Background and hypothesis

Despite recent advancements in the therapy against melanoma, metastatic dissemination remains a primary cause of patient mortality, and a clinically-unmet problem. During invasion, melanomas can switch from mesenchymal to amoeboid migration modes. Then, to disseminate through the blood vessels, melanomas must withstand ferroptosis lipid peroxidation cell death. Aggressive melanoma cells show a dedifferentiated phenotype and develop resistance to targeted therapies. However, metastatic aggressive melanoma cells are also intrinsically more sensitive to ferroptosis. What are the underlying molecular mechanisms and whether this represents a possible weak spot of melanoma remains unknown.
We recently found that RAC1, a main factor instructing melanoma therapy-resistance and invasive ability, also induces cell sensitivity to ferroptosis.

Aims

We propose: (i) to identify what is the cause of blood-induced FPT; (ii) to better understand the regulatory logic and molecular mechanisms underlying RAC1-induced FPT sensitivity; (iii) to broaden its significance within melanoma subtypes; (iv) to explore how melanomas evade blood-induced FPT, including the mesenchymal-amoeboid plasticity; (v) to test whether inhibition of parallel known pro-oncogenic RAC1 effector pathways can synergize with FPT induction, and whether it is possible to resensitize ameboid escaper cells to FPT.

Experimental design

We will study established models of human metastatic melanoma in vitro, and validate the significance for metastasis in mice. We will use hypothesis-driven and middle-size unbiased screens to shed new light on this process. We will perform gene expression, metabolomics and protein-protein interaction studies to understand the mechanisms and to obtain new targets for therapy. We will validate gene function by RNAi and using drugs that could be useful to translate our results towards applications.

Results

We identified RAC1 as an inducer of ferroptosis sensitivity across multiple cancer cell types, downstream of different genetic and epigenetic inputs. Activation of RAC1 promotes ferroptosis sensitivity by lowering the cells' antioxidant metabolism, and this regulation is intertwined, at the molecular level via IQGAP1 and KEAP1, with the ability of RAC1 to promote mesenchymal migration. This indicates the interesting possibility that ferroptosis and oxidative stress sensitivity represents a cost that melanoma cells need to "pay" to become invasive and migratory.

Conclusions

In conclusion, our findings may provide a long-sought explanation to the observation that even if RAC1 has a potent pro-migratory activity, RAC1 activating mutations are found at a low rate in metastatic cancers.

Federica Rossin **MFAG - 2022**

University of Rome Tor Vergata

Cancer Associated Fibroblasts in melanoma: cysteamine as a novel approach to target TG2-HSF1-Wnt axis

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Background and hypothesis

Tumors are complex multicellular systems characterized by dynamic and reciprocal interactions between cancer cells and components of tumor microenvironment (TME). Among the most prevalent cell types within the tumor stroma, cancer-associated fibroblasts (CAFs), possess the capacity to remodel the extracellular matrix and secrete cytokines and growth factors that support tumor development. Melanoma, the deadliest form of skin cancer, arises from the uncontrolled proliferation of melanocytes and ranks among the most frequently diagnosed malignancies.

Signaling pathways such as Heat Shock Factor 1 (HSF1) and Wnt have been identified as pro-invasive oncogenic drivers in human melanoma and are aberrantly activated in CAFs, contributing to tumor progression.

Our research has demonstrated that HSF1 activation is mediated by Transglutaminase 2 (TG2), an enzyme that catalyzes post-translational modifications of proteins. In addition, we found that TG2 is essential for proper induction of the Wnt signaling pathway, an effect that appears to occur via HSF1 regulation. Furthermore, pharmacological inhibition of TG2 using cystamine, an FDA-approved drug, impairs the activation of both HSF1 and Wnt signaling.

Aims

The aim of this project is to investigate whether TG2 promotes melanoma development through modulation of the HSF1-Wnt signaling axis in CAFs. Characterizing the TG2-HSF1-Wnt pathway within the TME will provide critical insights into the molecular mechanisms by which stromal fibroblasts are reprogrammed to acquire tumor-promoting properties, remodel the microenvironment, and sustain tumorigenesis.

Experimental design

We will perform 2D and 3D co-culture experiments of human and mouse melanoma cells with the CAFs to study how the modulation of the TG2-HSF1-Wnt axis in CAFs affects melanoma growth. We will investigate the use of cysteamine to inhibit the TG2-HSF1-Wnt axis in CAFs both in in vitro and in vivo mouse models with the aim to fully translate our results to a clinical approach.

Results

Using a 3D spheroid model, with both murine (B16F10) and human (A2058) melanoma cells, we found that cancer cells show an upregulation of TG2 transamidase activity, indicating that the enzyme is activated in melanoma cells. We also found that melanoma cells undergo to cell death after cystamine treatment, suggesting a potential toxic effect of the TG2 inhibitor on melanoma.

To evaluate how the modulation of the TG2-HSF1-Wnt axis in CAFs affects melanoma growth, we performed 3D co-culture of melanoma cells and human dermal fibroblasts (HDF) and we set up a procedure to obtain CAFs.

We confirmed that cystamine induces cell death just in melanoma cells and not in CAFs. Moreover, we observed that cystamine treatment is able to inhibit melanoma cell migration.
Interestingly, we demonstrated that cystamine also affects melanogenesis, through the inhibition of tyrosinase activity, a key enzyme involved in the melanin synthesis. Of note, increased melanogenesis has been correlated to melanoma progression.

Conclusions

In this first part of the project, we demonstrated that cysteamine is able to act by inhibiting the TG2 transamidase activity, which result constitutively active in melanoma and selectively triggers melanoma cell death and blocks migration. Moreover, cysteamine impairs the melanogenesis pathway, which is correlated to invasiveness and malignancy of melanoma.

Alberto Servetto **MFAG - 2022**

University of Naples Federico II

Exploring chromatin remodeling and transcriptional profiles causing resistance to CDK4/6 inhibitors in ER+ breast cancer.

Background and hypothesis

Despite an undoubted clinical benefit of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) in estrogen receptor positive (ER+) breast cancer (BC), resistance eventually occurs, causing tumor progression. DNA-Sequencing studies identified genomic abnormalities that did not fully recapitulate the landscape of resistance. Therefore, we explored the role in resistance to CDK4/6i of single-nucleotide variants (SNVs) of non-coding regulatory regions that affect binding of transcription factors (TFs), ultimately leading to changes in three-dimensional (3-D) chromatin landscape.

Aims

1) Dissecting 3-D chromatin landscape and SNVs responsible for palbociclib resistance; 2) Identifying TFs crucially involved in drug resistance and the role of FOXA1 and ERα signalling in palbociclib-resistant cells; 3) Modelling clinical resistance to CDK4/6i using ex vivo patient-derived organoids (PDOs).

Experimental design

We generated ER+/HER2- palbociclib-resistant T47D and MCF7 BC cells (T47D-PR and MCF7-PR), by exposing parental cells to increasing doses of the drug until resistance occurred. In T47D-PR, MCF7-PR and parental cells, we performed: 1) High-throughput Chromosome Conformation Capture (Hi-C), to investigate 3D chromatin remodelling associated with drug resistance; 2) Whole Genome Sequencing (WGS), to detect SNVs, insertions or deletions (InDels, <50 bp) acquired in PR cells; 3) ATAC-Seq to identify TFs potentially leading to drug resistance. Next, we established PDOs from metastatic BC biopsies collected from patients progressing on CDK4/6i.

Results

Hi-C identified 2,189 differential interactions between T47D and T47D-PR cells (FDR < 0.05). More in detail, 844 interactions were gained in T47D-PR cells. Instead, 1,345 were lost in T47D-PR cells. Similarly, we found 896 gained and 1,086 lost interactions in MCF7-PR cells, respectively. We intersected coordinates of differential chromatin interactions with list of candidate enhancers and promoters from cis-Regulatory Elements by ENCODE. We found that ~80% of all anchors of gained or lost interactions in PR cells are listed as enhancers, and ~20% as active promoters. In T47D-PR compared to T47D cells, Hi-C also revealed 356 gained and 1,406 lost topologically associated domains (TADs). 2,033 TADs were shared between two cells lines. Next, in MCF7-PR, compared to MCF7 parental cells, we found 498 and 783 gained and lost TADs, respectively. Instead, 3,874 TADs were shared.

In T47D-PR cells, we found 5,465 acquired SNVs and 5,248 InDels. Notably, 3,374/5,465 (61.7%) and 1,610/5,465 (29.4%) SNVs occurred at intergenic and intron genomic regions, respectively. Next, 2,695/5,248 (51.3%) and 2,137/5,248 (40.7%) InDels occurred at intergenic and intron regions, respectively. In MCF7-PR cells we found 7,386 acquired SNVs and 5,206 InDels. More in detail, 4,602/7,386 (62.3%) and 2,102/7,386

(28.5%) SNVs occurred at intergenic and intron regions. Finally, 2,629/5,206 (50.5%) and 2,165/5,206 (41.6%) InDels occurred at intergenic and intron loci, respectively.

ATAC-Seq revealed 5,223 (LogFC <-1) and 4,361 (LogFC >1) newly closed and open regions, respectively in T47D-PR compared to T47D cells. Next, in MCF7-PR compared to MCF7 cells, we found 948 and 1,625 newly closed and open regions, respectively. We intersected ATAC-Seq data with those from publicly available ChIP-seq datasets (Toolkit for Cistrome Data Browser). Newly open regions in PR cells were enriched for binding sites of FOS family members. Also, newly closed regions in PR cells revealed enrichment for ERα binding sites and other ERα-interacting TFs, such as FOXA1, GATA3, EP300 and GREB1. Coherent with these findings, PR cells exhibited reduced sensitivity to fulvestrant and higher estrogen-independent growth, compared to parental cells.

Finally, we have successfully established 9 PDOs cultures

Conclusions

SNVs and chromatin remodeling are involved in resistance to CDK4/6i. Further results of these studies may help to identify novel therapeutic vulnerabilities in ER+ BC

Elisa Taiana **MFAG - 2022**

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano

Investigation of the biological and molecular relevance of NONO protein in multiple myeloma

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Background and hypothesis

Multiple myeloma (MM) is a malignant proliferation of bone marrow plasma cells (BMPCs) with variable clinical outcome, which, despite treatment advances, still remains incurable. Long non-coding RNA NEAT1, the scaffold of paraspeckle (PS) organelle, has been linked to cancer development and progression, by playing a critical role in DNA repair and cell survival in MM. NONO, a protein involved in NEAT1 stability and PS assembly, is essential for several cellular functions and gene regulation. Notably, NONO is upregulated in BMPCs of MM patients, and its high expression level correlates with worse overall survival and progression free survival. In addition to its essential role within PSs, NONO may also have independent functions in MM cells.

Aims

To elucidate the biological and molecular functions of NONO in MM, with the goal of uncovering its potential role in disease pathogenesis. Exploiting next-generation sequencing, this study further aims to investigate how NONO influences the transcriptomic landscape of MM plasma cells and to delineate its functional contribution both within the context of PS and independently of it.

Experimental design

Gymnotic delivery of a specific LNA-gapmeR targeting NONO (gNONO) was employed to silence NONO expression (NONO-KD) in a panel of 4 human MM cell lines (HMCLs). Dose–response curves were generated using Trypan Blue exclusion assays, while cell viability was assessed via the Cell Counting Kit-8 (CCK-8) assay. IC_{50} values and drug interaction effects were calculated using CompuSyn software. Clonogenic potential was evaluated by methylcellulose assay. Cell cycle phases distribution and apoptosis induction were analyzed by flow cytometry (FACS). PS integrity was assessed by confocal microscopy through combined NEAT1 RNA fluorescence in situ hybridization (RNA-FISH) and NONO immunofluorescence (IF). Western blotting (WB) was performed to quantify PS proteins (PSPs) levels. RNA-seq libraries were prepared following Illumina Stranded TotalRNA PrepLigation with Ribo-zero Plus protocol (Illumina). Sequencing was performed on Illumina Novaseq 6000 S2 cartridge. CoMMpass data were retrieved from the Interim Analysis 15a (MMRF_CoMMpass_IA15a, accessed on 16 October 2020).

Results

To elucidate the specific role of NONO in MM, we employed a loss-of-function strategy using LNA-gapmeR antisense oligonucleotides delivered via gymnosis. HMCLs were treated with either gapmeRs targeting NONO (gNONO) or a non-targeting control (gSCR) at the time of seeding. All the 4 tested HMCLs, albeit at different levels, showed high sensitivity to NONO-KD starting from the 3rd day of gapmeR exposure, as revealed by the IC50 value (mean IC50 value = 6.5μM).

A sub-cytotoxic 5 μM gNONO dose was selected for further experiments, achieving >80% mRNA silencing efficiency across all HMCLs.

Cell viability assays (CCK-8) over 7 days revealed a significant reduction in the number of viable cells following NONO depletion. Colony formation assays further confirmed the reduced clonogenic potential in NONO-KD cells (range: 0–11 colonies, median = 3) compared to controls (range: 7–30, median = 25). May-Grünwald staining revealed characteristic signs of cellular distress, including cytoplasmic vacuolation and membrane blebbing. Consistent with this phenotype, flow cytometry showed altered cell cycle distribution and a ~2-fold increase in Annexin V-positive cells, indicating apoptosis onset from day 4 post-treatment.

Importantly, NONO depletion displayed synergistic anti-tumour effects when combined with standard MM therapies such as bortezomib, carfilzomib, melphalan, and olaparib, highlighting its therapeutic potential.

Western blot and immunofluorescence analyses revealed reduced NONO protein levels and upregulation of SFPQ and PSPC1, indicating compensatory regulatory mechanisms.

Additionally, NONO knockdown led to a 50–70% reduction in NEAT1 expression and disruption of PSs structure, underscoring NONO's critical role in preserving NEAT1 stability and PSs integrity also in MM cells.

Of note, in scramble cells, beside the NONO-NEAT1 co-localizing signals, we highlighted the presence of a diffuse NEAT1-independent NONO staining, thus reinforcing the hypothesis of its PSs-unrelated roles, which deserves further investigations.

To explore the role of NONO in both PS-related and independent contexts, we compared data from NONO-KD or NEAT1-KD AMO1 and LP1 cells; overlapping pathways between NONO-KD and NEAT1-KD conditions should be suggestive of the involvement of NONO in PS-related functions.

In NEAT1 and NONO silenced cells we highlighted a significant downregulation of gene sets associated with chromatin modifications, as well as pathways related to WNT/β-catenin and NOTCH signalling. These findings were further confirmed by analysing RNA-seq data from AMO1 cells, which we previously engineered to overexpress NEAT1 and PSs. This analysis revealed a significant positive modulation of the same pathways (NES ≥ 1.5, padj < 0.05). Further confirmation was obtained by stratifying samples from the CoMMpass dataset based on NONO expression levels, comparing the expression profiles between the two extreme quartiles, and conducting GSEA on the list of differentially expressed coding genes.

As mentioned above, since NONO is essential to prevent NEAT1 degradation, its silencing results in a marked downregulation of NEAT1 expression levels, thereby impacting the transcriptome of NONO-KD cells in a NEAT1-dependent manner. As a result, all the pathways modulated in the NONO-KD HMCLs were also confirmed in the NEAT1-KD samples, making it impossible to identify any pathways regulated by NONO independently of PSs. However, the analysis of data from the extreme quartile of NONO expression in the CoMMpass dataset enabled the identification of specific pathways not shared with NEAT1-KD HMCLs, which may suggest pathways that NONO regulates independently of PS in MM plasma cells. This analysis revealed NONO's involvement in RNA splicing or maturation, protein trafficking to the cytoplasm, as well as its role in mitochondrial biogenesis and cell-matrix adhesion.

Conclusions

Our findings demonstrate that NONO plays a critical role in the survival and proliferation of MM cells. Through both molecular and phenotypic analyses, we provide compelling evidence that NONO significantly contributes to PS integrity by stabilizing NEAT1 and regulating the expression of PSPs. The overlap in transcriptomic changes between NONO-KD and NEAT1-KD cells confirms that many of the NONO-related effects are mediated through PS disruption. However, integrative analyses using patient-derived datasets uncovered a distinct set of PS-independent functions for NONO, particularly RNA processing, mitochondrial regulation, and cell adhesion, highlighting its multifaceted role in MM biology.

Eleonora Vannini **MFAG - 2022**

CNR Istituto di Neuroscienze

CTX-CNF1 reprograms the glioblastoma immune microenvironment and enhances survival in preclinical models

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Background and hypothesis

Glioblastoma (GB) is the most aggressive primary brain tumor in adults, characterized by poor prognosis and limited treatment options. The blood-brain barrier (BBB) hampers drug delivery, and the tumor microenvironment (TME) is immunosuppressive, with poor T-cell infiltration and predominantly anti-inflammatory tumor-associated macrophages (TAMs). CNF1 is a bacterial toxin shown to inhibit glioma growth and stimulate immune responses, but it cannot cross the BBB. To overcome this, we engineered a chimeric protein, CTX-CNF1, combining CNF1 with Chlorotoxin (CTX), a glioma-targeting peptide that crosses the BBB.

Aims

1) To assess the therapeutic efficacy of CTX-CNF1 in immunocompetent murine glioma models.
2) To investigate its mechanism of action, focusing on immune cell involvement and macrophage reprogramming.
3) To test CTX-CNF1 in combination with immune checkpoint inhibitors (ICIs).
4) To evaluate the induction of long-term anti-tumor immune memory.

Experimental design

Models: Orthotopic glioma models (GL261 and CT-2A) in C57BL/6 mice.
Treatment: Intravenous CTX-CNF1 once weekly for 3 weeks, post-MRI diagnosis.
Assessments: Survival, tumor volume (MRI), motor function (Grip Strength, Grid Walk), immunofluorescence (CD8+, F4/80+), qPCR/ELISA on murine and human macrophages, tumor rechallenge, combination with anti-PD-1/PD-L1.

Results

CTX-CNF1 treatment markedly enhanced survival and reduced tumor burden in both GL261 and CT-2A glioma models. In the GL261 model, more than 50% of treated mice achieved complete tumor eradication and remained tumor-free for over 180 days. CTX-CNF1 promoted robust infiltration of cytotoxic CD8+ T cells into the tumor microenvironment, increasing expression of Granzyme B and Perforin. The therapeutic benefit was abolished by CD8+ cell depletion, underscoring the essential role of CD8+ T cells in mediating anti-tumor activity. Importantly, CTX-CNF1 did not directly activate T cells in vitro, indicating an indirect immunomodulatory mechanism. Both in vitro and in vivo data demonstrated that CTX-CNF1 reprograms tumor-associated macrophages (TAMs) toward a pro-inflammatory, M1-like phenotype. Notably, mice cured with CTX-CNF1 resisted glioma re-challenge, suggesting the development of durable anti-tumor immune memory. Finally, co-administration of CTX-CNF1 with anti-PD-1 immune checkpoint blockade further

extended survival, resulting in long-term remission in up to 75% of treated animals.

Conclusions

CTX-CNF1 is a promising immunomodulatory therapeutic for glioblastoma. It selectively targets tumor cells, reprograms the immune microenvironment, enhances CD8+ T cell-mediated responses, and induces long-term protection. Its synergistic effect with immune checkpoint blockade supports its clinical development as a treatment for GB.

PARTICIPANTS

Manuel Albanese **MFAG - 2023**

Fondazione "Istituto Nazionale Genetica Molecolare - INGM"

Engineered Extracellular Vesicles for personalized leukemia therapy

Background

During the last few years, the treatment of cancer patients has been revolutionized by modern oncology, with a deeper understanding of cancer cells at the molecular level moving forward to personalized medicine. The classic broad anti-cancer drugs are now often combined with more tailor-made treatments (e.g.immunotherapy), to target each cancer type more precisely and with increased efficacy. Despite current advances, there is still a need for more specific targeted therapies, a long-sought goal to target tumors for complete eradication.

Hypothesis

The ideal treatment effectively and specifically targets cancer cells, limiting possible side effects. Extracellular vesicles (EVs) are promising carriers of anti-cancer drugs to achieve this goal. However, two main problems are currently limiting the use of EVs for therapy: low cargo delivery of EVs into target cells and low enrichment of the therapeutic protein of interest into EVs. Here, we hypothesize that, if ad hoc engineered, EVs can be loaded with any cargo protein and re-directed against any specific target cell.

Aims

This project aims at generating a platform of engineered EVs (eEVs) with multiple features to specifically target different cancer cells. Acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL) are selected in this proposal as proof-of-concept model to test our hypothesis. ALL and CLL have been chosen due to their lymphocytic nature, their main localization in the bloodstream, and the easy access to patient samples. To monitor EV cargo delivery into target cells, we recently established a novel EV fusion assay. eEVs selected by fusion assay to be able to enter ALL and CLL will then be loaded with CRISPR/Cas9 ribonucleoproteins that carry guideRNAs to target leukemia-specific genomic alterations.

Experimental design

Engineered EVs will be designed with envelopes from different lymphotropic viruses (e.g. EBV and HIV-1) in combination with antibodies specific to antigens expressed by leukemia cells and with fusogenic molecules, to fuse efficiently to the target cells. For the cargo delivery, we will use a tunable self-cleavage-protease system in which Cas9 is fused with CD63 that confers enrichment into EVs, and with a protease capable of cleavage in cis, allowing cargo release into the target cells. To better control the Cas9 delivery, the system can be easily fine-tuned by a specific protease inhibitor.

Expected results

We will generate a list of eEVs that will cover specificity for ALL and CLL from different patients. eEVs will first be challenged against in vitro and ex vivo models of leukemia to improve EVs delivery and anti-tumor effector function. Additionally, the most effective anti-ALL eEVs will be validated in vivo in a ALL patient-derived xenograft (PDX) model. Once established, the platform will consist of a combination of eEVs with

different targeting/fusogenic machineries and Cas9-gRNAs, capable of targeting different genomic alterations in a tumor and patient-specific manner.

Impact on cancer

This novel therapeutic approach is going to revolutionize current cancer treatments. eEVs will be a new class of extremely flexible drugs that can be adapted for each type of malignancy and to each patient, providing a completely personalized and precise therapeutic option to replace or be combined with already available treatments.

Valentina Audrito **MFAG - 2021**

Università degli Studi del Piemonte Orientale "Amedeo Avogadro"

NAMPT as a driver of melanoma progression and immune evasion: therapeutic target for novel combination therapies

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Background

Targeted-therapy and immune checkpoint inhibitors (ICIs) have notably improved the treatment of BRAF-mutated metastatic melanoma (MM) patients; however resistance to treatment dramatically impacts on the survival of patients, indicating that complementary/alternative therapeutic approaches are needed to obtain long-lasting remission. Resistance to conventional therapies is coupled by a set of rewiring processes in both tumor and immune cells, regulated by the BRAF oncogenic signaling and environmental inputs.

We demonstrated that BRAF(i)nhibitors-resistant MM requires increased amounts of NAD, an essential redox cofactor and signaling molecule, achieved by selective over-expression of the rate-limiting enzyme nicotinamide phosphoribosyltransferase (NAMPT). NAMPT become a driver of melanoma progression and resistance to targeted-therapy. In addition, is highly released in MM patients' plasma correlating with tumor burden. Furthermore, NAMPT inhibition decreased cell survival and reduced melanoma tumor growth, suggesting that it may be therapeutically targeted.

Hypothesis

The innovative hypothesis behind this project is that, in addition to promote BRAFi/MEKi resistance, NAMPT may be also a key regulator of response to ICIs in MM, linking NAMPT-dependent metabolic reprogramming and immune regulation.

Aims

This proposal aims at dissecting the mechanistic basis of NAMPT functions in BRAF-mutated MM addressing i) its intrinsic oncogenic properties modulating cancer metabolic/epigenetic/transcriptional reprogramming, acting in the cytosol but also as nuclear protein bound to chromatin, and regulating melanoma immune escape mechanisms leading to ICIs resistance; ii) its role as soluble factor, acting on melanoma cells themselves, as well as affecting immune responses within the melanoma microenvironment contributing to immune evasion. For these reasons, NAMPT may be druggable at multiple levels [enzymatic activity; extracellular (e)NAMPT functions], opening the way to novel combination therapies to overcome drug resistance. Therefore, the last goal of the project aims at providing preclinical validation of the therapeutic impact of NAMPT-targeting strategies used in combination with ICIs.

Experimental design

The project is organized in 6 work-packages (WP). Combining innovative molecular and "omics" techniques and a solid experimental expertise of functional assays in vitro/in vivo, we will plan to deeply examine the regulation of NAMPT expression, as well as its post-transcriptional modifications and of sub-cellular and intra/extra-cellular trafficking mechanism. Then we will address the multiple NAMPT functions as intracellular/nuclear protein but also as soluble factor conditioning tumor-immune cell crosstalk. Lastly, xenograft MM models in mice will be exploited to test the efficacy of NAMPT-targeting in combination with

ICIs.

Expected results

The ambition of the project is to shed light on i) nuclear NAMPT chromatin-bound fraction role possibly linking metabolism and epigenetics; ii) functional role of NAMPT as soluble factor within melanoma microenvironment; iii) NAMPT-dependent potential immune suppressive functions; and iv) NAMPT-targeting strategies in combinantion with ICIs.

Impact on cancer

This project will dissect the multiple faces of NAMPT in MM as well as the impact of its manipulation in regulating intrinsic properties of tumors, but also modulating immune responses. Lastly, the most translational and long-term area of this project concerns the therapeutic potential of NAMPT-targeting in the current landscape of melanoma drugs, with an eye to possible future application also in other tumors.

Rosario Avolio **MFAG - 2024**

Università degli Studi di Napoli "Federico II"

Uncovering new vulnerabilities of ovarian cancer pharmaco-resistance via unbiased identification of RNA-binding proteins

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Background

RNA-binding proteins (RBPs) control every step of cellular mRNA life cycle, strongly contributing to post-transcriptional gene regulation. A handful of studies have described the role of RBPs in high-grade serous ovarian cancer (HGSOC) drug-resistance; yet most of these studies have been carried out in experimental models not resembling HGSOC features and lack sufficient mechanistic insights to propose a realistic therapeutic strategy. To fill this gap, we applied a proteomic-based approach for the unbiased identification of RBPs potentially involved in HGSOC drug-resistance. Our results show about 1500 putative RBPs, including unconventional RBPs like metabolic enzymes, displaying changes between platinum-sensitive and platinum-resistant HGSOC cells, pointing towards a remodeling of RBPs dynamic during the acquisition of platinum-resistance.

Hypothesis

Our long-term goal is to understand the contribution of post-transcriptional gene regulation to the onset of drug-resistance in reliable HGSOC models to unveil novel therapeutic targets and strategies for the management of this disease. To achieve this goal, the objective of this proposal is to comprehensively characterize RBPs and related RNA-targets in sensitive and resistant HGSOC models. Our central hypothesis is that changes in RBPs expression and/or activity, responsible for dysregulation of associated post-transcriptional networks, could promote drug-resistance in HGSOC.

Aims

We propose the following Aims to address our hypothesis:

- Assess the relevance of selected RBPs to the onset of drug-resistance in HGSOC models' representative of different status of chemosensitivity;
- Identify dysregulated RBP-dependent post-transcriptional signatures responsible for chemoresistance acquisition;
- Design RNA-metabolism targeting approaches and evaluate their stand-alone anti-tumoral effect and the potential sensitizing activity towards conventional chemotherapy.

Experimental design

The project is organized in four Specific Aims. Aim 1 will evaluate the ability of RBPs to influence response to conventional therapies both in HGSOC cells and patient derived organoids (PDOs). Aim 2 will validate the RNA binding ability of the selected top hits and the correlation between RNA-binding activity and HGSOC chemoresistance. Aim 3 will identify RBP-dysregulated post-transcriptional networks responsible for development of HGSOC pharmaco-resistance by RNA-seq and RIBO-seq. Finally, Aim 4 will be dedicated to the design of novel therapeutic strategies, based on the results obtained from the previous tasks, and their testing both in vitro (HGSOC cells and PDOs) and in vivo (xenograft models).

Expected results

Starting from the screening of RBPs showing different dynamic between platinum-sensitive and platinum-resistant HGSOC cells, this project is expected to: 1) provide the first evidence of RNA-binding ability for novel, putative identified RBPs and their tumorigenic potential; 2) identify post-transcriptional signatures responsible for the establishment of drug-resistance in HGSOC; 3) design RNA therapeutic strategies for the treatment of resistant HGSOCs.

Impact on cancer

We think that the proposed research is significant because it will greatly advance our understanding of the molecular mechanisms responsible for the establishment of drug-resistance potentially leading, in a long-term view, to the development of new routes of therapeutic intervention for the management of HGSOC. Results from this project will not only have a possible strong positive impact on patients' health, but it will also have an economic impact, supporting research investments in novel promising fields of study.

Isaia Barbieri **Start-Up - 2021**

Università degli Studi di Torino

Investigating RNA methylation in cancer

Background

More than 100 different types of RNA chemical modifications are known to exist. Despite being known for several decades their functions have only recently been deeply investigated.

The new field of epitranscriptomics uncovered many different new functions of RNA modification both in normal physiology and disease. They are involved in all aspects of RNA biology, including stability, processing, structure, localisation, and translation. Modifications of RNA are mediated by specific enzymes, while separate enzymatic processes mediate their removal and downstream effect.

Many studies found that RNA modifications and their catalytic enzymes are often dysregulated in cancer and could represent a new class of drug targets for cancer therapeutics.

Hypothesis

Our hypothesis is that specific RNA modifications and the enzymes responsible for their deposition can drive cancer cell proliferation and cancer progression while they are dispensable for the survival of normal, untransformed cells. Consequently the activity of RNA modifying enzymes could be targeted for the treatment of specific cancer types. Specifically, we will study two different types of RNA methylations, m6A and m2,2,7G in the context of Anaplastic large cell lymphoma and acute myeloid leukaemia respectively. A third research line will identify RNA modification enzymes involved in breast cancer progression.

Aims

The three main aims of the proposed project correspond to our three main lines of research:

1: Study METTL3 inhibition in ALK-driven malignancies.

2: Characterize TGS1-dependent cap hypermethylation in leukemia.

3: Identify RNA enzymes involved in breast cancer progression.

Experimental design

We will use a combination of hypothesis-driven and unbiased approaches to understand the functions of RNA enzymes in cancer. The first approach, based on our previous studies, is aimed at characterizing the role of specific RNA enzymes in specific cancer types. The second approach is based on CRISPR-Cas9 dropout screens to identify RNA enzymes specifically required for the growth and progression of cancer cells.

In addition to our phenotypic characterization of cancer cells upon RNA methyltransferase inactivation we will also investigate the molecular mechanisms involved by mapping modifications transcriptome-wide and identify direct targets of RNA enzymes in cancer. We will achieve this by using several high throughput techniques such as RNA-seq, RIP-seq and mass spectrometry.

Expected results

At the end the proposed project, we expect to have identified roles and therapeutic value of specific RNA modifying enzymes in specific cancer types. In particular, we will have identified the best strategy to target

m6A enzymes in ALK-driven lymphomas and lung cancer. We will have characterized the role of TGS1 in regulating the metabolic state of acute myeloid leukaemia cells and determined its therapeutic value. Finally, we will have identified RNA enzymes driving cell proliferation and tumour progression in triple negative breast cancer cells and characterized their functions.

Impact on cancer

Our studies will provide new potential therapeutic options for the treatment of different cancer types and will provide translational and clinical studies aimed at developing and testing small molecule inhibitors targeting RNA enzymes as cancer treatments. Additionally, we will discover and characterize new molecular mechanisms and biological pathways involved in tumorigenesis, tumour progression and drug resistance.

Alessandro Bertucci

Università degli Studi di Parma

CRISPR-Cas-Powered Detection Technologies for Protein Biomarkers in Bodily Fluids [CRISPOWER]

Background

Early diagnosis of tumors is essential for enhancing patient outcomes and optimizing healthcare resources. Protein biomarkers play a crucial role in this process due to their involvement in key biological processes and their altered expression during various stages of cancer. Monitoring these biomarkers can guide therapeutic strategies and track disease progression. However, current detection technologies such as ELISA, CLIA, ECLIA, and LFIA face limitations in sensitivity, specificity, cost, and usability, especially for point-of-care (POC) applications.

Hypothesis

Originally discovered as part of the microbial adaptive immune system, CRISPR-Cas systems have been extensively engineered for genome editing and, more recently, for diagnostics of nucleic acids. Leveraging CRISPR-Cas technology for protein detection can overcome the limitations of conventional methods. CRISPR-Cas systems, particularly CRISPR-Cas12a and CRISPR-Cas13, offer significant signal amplification capabilities due to their collateral cleavage activity upon target recognition. Integrating DNA nanotechnology with CRISPR-Cas systems can translate the presence of protein biomarkers into nucleic acid signals, enabling highly sensitive, rapid, and cost-effective diagnostic tools suitable for POC applications.

Aims

This project aims to develop innovative CRISPR-Cas-powered detection technologies for cancer protein biomarkers in clinical samples. The specific objectives are: 1) Demonstrate proximity-based mechanisms for CRISPR-Cas12a activation using DNA nanotechnology. 2) Develop CRISPR-Cas12a-powered optical assays for precise quantification of protein biomarkers. 3) Engineer lateral flow assays (LFAs) incorporating CRISPR-Cas signal enhancement for rapid POC protein detection.

Experimental design

The project will utilize DNA nanotechnology to create proximity-based mechanisms for CRISPR-Cas12a activation in response to specific cancer biomarkers. By designing nucleic acid-based nanodevices conjugated with protein-binding moieties, binding events will be converted into inputs for CRISPR-Cas12a activation, generating amplified fluorescence signals for detection. Key biomarkers targeted include PSA, AFP, CEA, CA19.9, PDGF, and VEGF. Initial validation will be conducted using spiked serum samples to determine analytical characteristics, followed by translation into a lateral flow-based platform for rapid visualization and quantification of target protein levels at the POC.

Expected results

The CRISPR-Cas12a-powered assays are expected to offer enhanced sensitivity and specificity in detecting cancer biomarkers, enabling earlier and more accurate diagnosis. The assays will be designed to be cost-

effective and rapid, making them suitable for low-resource settings and POC testing. The platform will be versatile, capable of detecting various biomarkers by modifying the DNA probes, demonstrating broad utility in oncology diagnostics and prognostics.

Impact on cancer

This research aims to advance the state of cancer diagnostics by providing highly sensitive, rapid, and cost-effective tools for early diagnosis and monitoring of disease progression. The development of fluorescence-based assays and user-friendly LFAs with optical readouts will enable real-time monitoring of cancer biomarkers, improving patient management through immediate clinical decision-making. By translating sophisticated CRISPR-based detection mechanisms into molecular assays and practical POC tools, this project will contribute to better patient outcomes and pave the way for future breakthroughs in CRISPR-powered diagnostics and DNA nanotechnology in cancer research.

Valentina Biagioli **MFAG - 2024**

Alma Mater Studiorum Università di Bologna

Disparities in cervical cancer prevention and early detection among immigrants in Italy (DCIMIT)

Background

Proactive prevention and early detection are crucial in the fight against cervical cancer, with Human papillomavirus (HPV) being the primary cause. Immigrant women (foreign-born) in Italy have a higher risk of cervical cancer (OR=3.54) but lower participation in HPV screenings (72% vs. 78%). This leads to a greater burden of cervical cancer among immigrant women in Italy. It is crucial to address disparities by providing culturally sensitive interventions for cervical cancer prevention for immigrants across Italy.

Hypothesis

We believe that (1) immigrants have lower participation in cervical screening and receive lower quality care due to various barriers (e.g., language, cultural, and socio-economic status), which should be further explored in terms of their importance and impact; (2) incidence and mortality of cervical cancer in immigrants are higher than in non-immigrant population; and (3) a community-based intervention could improve immigrants' participation in cervical cancer screening.

Aims

This project aims to address disparities in cervical cancer screening among immigrants in Italy. Specific aims are: (1) To explore the barriers to early detection of cervical cancer among immigrant women diagnosed with cervical cancer; (2) To analyze data from cancer registries and measure cancer burden by comparing the incidence, mortality, and survival rate of cervical cancer between immigrants and non-immigrants in Italy; (3) To design and implement a community-based intervention to improve immigrants' participation in cervical cancer screening.

Experimental design

Overall, we plan to conduct three sub-studies: (1) a mixed-methods study with a two-phase explanatory design involving a sequential collection of quantitative (n=300) and qualitative (n=60) data from immigrant women with cervical cancer who are receiving care at ten clinical centers in Italy; (2) a register-based study using cancer registry data on cervical cancer in various regions of Italy; and (3) a community-based interventional study to improve participation in cervical screening for healthy immigrant women aged 25 to 65 living in Rome (n=205); they will be compared to immigrant women living in Milan, who will serve as the control group.

Expected results

The mixed-methods study will identify reasons for unequal access to care and participation barriers in cervical screening among immigrants. Additionally, we will provide an overview of the accessibility of cervical cancer prevention and care services for immigrant women. Based on the register-based study, we will provide a comprehensive assessment of the gap in cervical cancer incidence and survival between

immigrants and non-immigrants. Based on the interventional study, an educational program will be developed to increase cervical screening participation by 15% in the intervention group compared to the control group.

Impact on cancer

Our community-based intervention model has the potential to be implemented nationally in the future, reducing the burden of cervical screening among all immigrant women in Italy. Overall, this project will reduce the cancer burden among immigrant women by enhancing their participation in cervical cancer screening programs. It will also pave the way for conducting research and mitigating disparities of other cancer types among immigrants in Italy.

Giulia Biancon **Start-Up - 2023**

Fondazione I.R.C.C.S. Ca' Granda Ospedale Maggiore Policlinico

The oncogenic role of biomolecular condensates in splicing factor-mutant myeloid malignancies

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Background

Among genetic aberrations responsible for ineffective hematopoiesis in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), somatic mutations in splicing factors (SFs) such as U2AF1 and SRSF2 are of significant interest as they are early occurring, frequent and associated with poor clinical outcome. However, the molecular mechanisms ultimately promoting cancer remain to be fully elucidated.

I recently performed multi-omics integration of RNA binding, splicing and turnover in U2AF1 S34F and Q157R mutant cells. For the first time, I demonstrated that U2AF1 mutations directly alter the biology of biomolecular condensates (BMCs), specifically increasing the availability of stress granule (SG) components. Immunofluorescence (IF) imaging and single-cell RNA-seq confirmed SG upregulation in U2AF1-mutant cell lines and primary MDS/AML samples. Of note, enhanced SG formation was associated with improved cell fitness under stress, which was reverted when using an SG inhibitor.

Finally, subsequent preliminary analysis pointed out over-representation of SGs in SRSF2 P95H mutant cells and alterations in P-bodies (PBs) and Cajal bodies (CBs) in U2AF1 S34F mutant cells.

Hypothesis

Changes in biomolecular condensates may therefore represent a unifying mechanism responsible for cancerous phenotype of hematopoiesis in MDS and AML.

Aims

In this project, my team and I will apply a multi-omics approach to investigate dysregulations in biomolecular condensates and to define their clinical relevance in U2AF1-mutant and SRSF2-mutant MDS/AML. The research strategy is specifically organized in the following aims: AIM 1. Dissection of SG perturbations at protein and RNA level; AIM 2. Evaluation of changes in other BMCs; AIM 3. Assessing therapeutic potential of targeting spliceosome and BMCs.

Experimental design

First, to simultaneously profile changes in SG proteins and RNAs as planned in AIM 1, we will perform SG purification in SF-mutant vs WT cells followed by high-throughput high-resolution proteomics and transcriptomics techniques. We will also investigate changes in SG ultrastructure by super-resolution microscopy and we will map alterations in SG interactions with spatial resolution. Then, to assess the effect of SF mutations on the assembly of PBs and CBs as planned in AIM 2, we will combine imaging data with transcriptomics and proteomics data comparing SF-mutant vs WT cells. Finally, within the translational AIM 3 we plan to: evaluate the effect of spliceosome modulators on BMCs by IF imaging, assess the anti-leukemic effect of SG inhibitors as monotherapy or combination therapy, perform small molecule screenings and gene silencing to identify and test PB and CB modulators.

Expected results

The results generated within this project will dissect the relationship between SF mutations and changes in BMCs, opening a new avenue in the study and in the treatment of SF-mutant myeloid malignancies.

Impact on cancer

SF mutations are enriched in MDS/AML but are also recurrent in many other cancers where aberrant splicing patterns promote and maintain transformed cells. SF mutations affect the processing of numerous RNAs and proteins and BMC dysregulation could reflect this collective mechanism. The studies here proposed will provide the basis for the design of tailored clinical trials aimed at improving patient survival in cancer.

Eduardo Bonavita **Start-Up - 2021**

Humanitas Mirasole S.p.A.

Analysis of mechanisms of immune evasion in liver cancer

Background

Hepatocellular carcinoma (HCC), the most prevalent form of liver cancer, represents the fourth cause of cancer-related death worldwide. Although HCC treatment has improved in the last years, current therapeutical options remain limited. Immune checkpoint blockade (ICB) therapies have revolutionised cancer treatment, showing encouraging results also in HCC. However, durable and complete responses in HCC patients are rarely observed. Further fundamental research is needed to understand the mechanisms underlying response to ICB, or lack thereof, to improve treatment efficacy.

Hypothesis

The hepatic microenvironment is a site of complex immunological activities. The tolerogenic nature of the liver microenvironment, can act as a barrier to anti-tumour immunity, foster cancer progression and resistance to immunotherapy.

Dendritic cells (DC) play a critical role in the maintenance of liver tolerance and in anti-tumour immunity. In particular, I hypothesis that a newly identified CCR7+ IL12+ DC population (termed mDC), found within many human tumours and characterised by a potential immunomodulatory function, exerts a central role in the regulation of anti-tumour immunity in the liver.

Aims

The primary aim of this proposal is to unveil the cellular and molecular mechanisms controlling immune responses during HCC carcinogenesis. This investigation will dissect the ability of mDC to orchestrate anti-tumour immune responses, their crosstalk with other prevalent liver immune cells, such as natural killer cells, T cells and macrophages and the regulation of liver tolerance. Moreover, the prognostic utility of mDC prevalence in human liver cancer will be tested. Finally, a liver tumour explant platform will be developed to functionally characterise the immunoregulatory properties of mDC in patients ex vivo.

Experimental design

The proposed research programme will make use of cutting-edge technologies combining autochthonous mouse models of liver cancer with analysis of human samples. Two newly generated mouse models will enable mDC monitoring or inducible depletion. Advanced flow- and mass-cytometry, imaging, biochemistry and single cell RNA sequencing will unveil the molecular and cellular mechanisms by which HCC cells regulate immunity, dissecting the factors regulating the balance between tolerance and anti-tumour immunity. Finally, the translational potential of the in vitro and in vivo experiments will be demonstrated with histopathological, clinical, in silico and ex vivo analysis of human tumours.

Expected results

The extensive characterisation of the hepatic microenvironment will deepen our understanding of the mechanisms orchestrating liver immunity, addressing the potential role of mDC in controlling spontaneous

and therapy-induced anti-tumour immune responses in liver cancer.

Impact on cancer

The successful outcome of this project will have important implications for the clinical management of liver cancer patients. It will inform the design of novel therapeutical strategies, of clinical protocols for patient selection and the discovery of potential biomarkers of ICB response.

Elisa Caiola **MFAG - 2021**

Istituto di Ricerche Farmacologiche "Mario Negri" I.R.C.C.S.

A platform of patients-derived models to find the best ERK inhibitor-based combinations in LKB1 mutated NSCLCs

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Background

Among non-small-cell lung cancers (NSCLCs), LKB1-mutated tumors represent an extremely aggressive subgroup. Patients affected by these malignancies completely lack at present a specific therapy (targeted therapies and immunotherapy) being LKB1 mutations mutually exclusive with targetable alterations and negative determinants of immune checkpoint inhibitors response.

We recently demonstrated in several preclinical models that LKB1 null NSCLCs are exquisitely sensitive to ERK inhibitor (ERKi) treatment. In addition, when PI3K/Akt/mTOR pathway alterations co-occur and limit ERKi efficacy, the combination of ERKi and inhibitors of the axis resulted successful. However, being NSCLC a very heterogeneous disease, we expected that other co-alterations could hamper ERKi efficacy.

In this context, 3D patient-derived preclinical models have been emerging as very useful tools that mimic better than 2D cell lines tumor architecture and heterogeneity.

Hypothesis

We hypothesized that ERKi-based treatments (including combinations with Pi3K/Akt/mTOR pathway) could represent a specific therapy for LKB1ness patients and 3D cultures (cell line- and patients-derived) could be the best models to obtain more translatable results.

Aims

The overall aim of the proposal is to identify the best ERKi-based combinations specifically active in LKB1 mutated NSCLCs. The project will be organized in four work packages (WPs), developed following four major sub-aims:

i) to enrich patients-derived xenografts (PDXs) and PDX-derived organoids (PDXOs) collection;

ii) to dissect the molecular mechanisms underlying the synthetic lethality between LKB1 loss and the pharmacological inhibition of ERK;

iii) to validate the results obtained from two FDA approved drugs library screenings in different 2D and 3D in vitro models with wt or deleted LKB1 protein;

iv) to translate the most promising results in the in vivo setting.

Experimental design

WP1. We will proceed generating of PDXs and PDXOs, starting from human tumor samples. The models will be histologically and molecularly characterized and matched with the primary tumor resected from the patient.

WP2. We will clarify the mechanisms underlying the exceptional response to ERKi observed in LKB1 deleted cells, by focusing on the regulation of RSK activation. We will apply different mass spectrometry-based experiments, aimed at analyzing the post-translational modifications on RSK protein and the phosphorylation status of all the LKB1 target kinases as candidates of RSK modulation, having excluded a

direct interaction with LKB1.
WP3. We will perform independent cell viability assays by treating a panel of LKB1 mutated and wt NSCLC cell lines first and 3D spheroids and PDXOs next with the candidate combinations. We will then characterize the combinations active in the LKB1 deleted 3D systems by performing molecular analyses on activation/inhibition of the proteins involved in the response to the drugs. Finally, 3D systems will be ad hoc modified (knock out/Knock in) to further validate the results.
WP4. The most promising results coming from WP2 and WP3 will be exploited in vivo performing antitumor activities on re-implanted PDXOs and on the correspondent PDXs.

Expected results

We will identify the best ERKi-based combinations possibly active in LKB1 mutated NSCLCs.

Impact on cancer

We will give a concrete chance to the undruggable LKB1ness patients subgroup to receive personalized and active therapies.

Luisa Carbognin **MFAG - 2020**

Fondazione Policlinico Universitario Agostino Gemelli IRCCS

Prognostic and predictive role of cyclin-dependent kinase 4/6 pathway in breast cancer with lobular histotype.

Background

Invasive lobular cancer (ILC), represents the second most common type of breast cancer after invasive ductal carcinoma (IDC). The lack of prospective studies conducted in tumors with lobular histology (5-15% of all invasive breast tumors), has led oncologists to use the same prognostic factors commonly used in ductal tumors in clinical practice, and, consequently, to treat ILC patients as IDC, despite the overall differences in terms of clinical-pathological features and outcome. Thus, the molecular characterization of ILC, and its integration with clinical and pathological predictors of outcome, currently represents a research priority, in order to more precisely stratify ILC patients according to prognosis and to predict their potential susceptibility to specific targeted treatments.

Hypothesis

In order to explore the molecular characterization of 'pure' ILC, the current proposal will explore 2 research hypotheses: 1) the genomic abnormalities of the CDK4/6 pathway may be prognostic in ILC patients receiving endocrine therapy (ET); 2) the dysregulation of the CDK4/6 pathway may predict resistance to CDK4/6 inhibitors (CDK4/6i) in metastatic LC patients treated with CDK4/6i plus ET. These hypotheses are supported by: 1) our preliminary results, suggesting that copy number gain of CDK4 (or alterations in CDK4/6 pathway) may negatively influence LC prognosis; 2) early findings indicating that genomic alterations in CDK4/6 pathway seem to be associated with resistance to CDK4/6i and ET.

Aims

The specific aims of the current project will allow:

1) To evaluate the impact of molecular factors (with particular regard to CDK4/6 pathway) in determining the prognosis of ILC patients;

2) To characterize and validate the previously identified prognostic molecular drivers in the pre-clinical setting (ILC cell lines and mouse models), exploring also the potential therapeutic efficacy of a targeted approach with CDK4/6i;

3) To evaluate the predictive impact of molecular alterations (involved in the cell-cycle regulation) in metastatic LC patients treated with endocrine therapy plus CDK4/6i.

Experimental design

The project will be performed through 3 phases (Work-Packages):

1) Retrospective collection of ILC samples with clinical-pathological annotations in different disease setting, in order to perform genomic/transcriptomic analysis with next generation sequencing;

2) Establishment of ILC cell culture and orthotopic mouse model of human ILC for in vitro and in vivo assessment of the identified prognostic molecular drivers and the potential therapeutic efficacy of a pharmacological approach;

3) Conduction of a prospective, proof of concept biomarker-stratified study in metastatic ILC patients, candidates to receive a first-line treatment with ET plus CDK4/6i. Patients' tissue and blood samples will be collected and analyzed by integrated genomic and proteomic analyses.

Expected results

The expected results will be:

1) To validate the potential prognostic role of CDK4/6 pathway alterations in ILC; 2) To identify predictive factors of resistance/response to CDK4/6i plus ET in metastatic LC patients.

Impact on cancer

This project may contribute to fill a need for clinical practice represented by the identification of predictors of prognosis and treatment efficacy for ILC to assist in the choice of a pertinent therapeutic decision.

Nicoletta Caronni **MFAG - 2024**

Università Vita-Salute San Raffaele

Targeting inflammation to restore anti-tumor immune response in pancreatic cancer

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Background

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with limited response to conventional and innovative therapies, including immunotherapies. The ineffectiveness of the immunotherapies is primarily attributed to the tumor microenvironment (TME) of PDAC, which is characterized by a fibro-inflammatory stroma with abundant fibroblasts and tumor-associated macrophages, and a low content of cytotoxic immune cells, such as CD8+ T cells and dendritic cells (DCs). DCs are critical for initiating and regulating anti-tumor immune responses. However, in PDAC, DCs become dysfunctional early in tumorigenesis, and their dysfunction is associated with an increase in inflammatory mediators within the TME, which favors PDAC development.

Hypothesis

We hypothesize that during the early stages of PDAC development, tumor-derived inflammatory signals drive immune evasion by hindering DC functions and trafficking abilities. We propose that inflammatory reprogramming of fibroblasts alters the interactions between DCs, fibroblasts, and PDAC extracellular matrix (ECM), thereby impacting DC fucntionalities.

Aims

The aim of the project is to elucidate the impact of inflammatory factors on DC functions during the early stages of pancreas tumorigenesis and to develop strategies for restoring DC-mediated anti-tumor immunity. The project is divided into three work packages (WPs):

WP1. To investigate the impact of inflammatory signals on DC functions during PDAC development.

WP2. To elucidate the impact of inflammatory signals on fibroblasts and ECM during PDAC development.

WP3. To restore DC-mediated anti-tumor immunity in PDAC.

Experimental design

We will integrate state-of-the-art transcriptomic and proteomics analyses with well-established in vivo and ex vivo models of PDAC and pancreatitis. Single cell RNA-sequencing and proteomic analyses will identify key regulators of DC functions as well as molecular interactions between DCs, fibroblasts and ECM during PDAC development. The identified regulators of DC functions will be targeted in bone marrow-derived DCs either by gene disruption or over-expression, depending on the role of the target molecules in DC activity. Using ex vivo and in vivo models of PDAC, we will evaluate the efficacy of engineered DCs in restoring anti-tumor immunity and controlling tumor growth.

Expected results

This project aims to uncover how inflammatory signals promote DC dysfunction, contributing to early immune evasion in PDAC. We will identify gene programs, molecular regulators, and soluble factors

associated with DC dysfunction during tissue damage and tumor initiation. Additionally, this research will elucidate the interactions between DCs and the TME, specifically cancer-associated fibroblasts and ECM components that might alter DC activation and trafficking. By targeting the most promising molecular candidates, we aim to develop strategies to restore antigen presentation and enhance anti-tumor immunity in PDAC.

Impact on cancer

This project introduces a groundbreaking approach for understanding and targeting immune evasion mechanisms in PDAC. By identifying key pathways and molecular mechanisms that lead to DC dysfunction in PDAC, we aim to develop new strategies to restore the antigen-presenting and immune-stimulating functions of DCs, which are crucial for effective anti-tumor immunity. The successful completion of this project will pave the way for novel therapeutic interventions for one of the most lethal malignancies.

Debora Carpanese **MFAG - 2023**

IOV - Istituto Oncologico Veneto - I.R.C.C.S.

Hyaluronan as an effective immunological adjuvant for the creation of protein-based vaccines against HER2 breast cancers

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Background

Protein-based vaccines represent a balance between a good safety profile and a relatively rapid manufacturing, but require efficient adjuvants to overcome their weak immunogenicity. We recently demonstrated and patented that the conjugation of protein antigens (Ags) to fragments of hyaluronan (HA) likely acting as toll-like receptor (TLR) 2/4 agonists, leads to robust and long-lasting Ag-specific immune responses in different mouse strains and in both infant and aged mice, suggesting that this technology might be particularly advantageous for poorly responding subjects such as cancer patients.

Hypothesis

Overexpression of the human epidermal growth factor receptor 2 (HER2) occurs in 15-30% of breast cancer (BC) cases and is considered an adverse prognostic factor, with 20% of patients with HER2+ early BC experiencing relapse and developing advanced disease. Anti-HER2 vaccines could be employed in a therapeutic setting alone or in combination with other therapies, to target early stages of disease or to prevent the development of metastases.

Aims

Willing to exploit the potentiality of our HA-based vaccination approach in the context of a real disease model, we aim at developing and studying the efficacy and safety of HA-conjugated protein and peptide-based candidate vaccines against HER2-expressing BCs, as well as elucidating the mechanism of action underlying HA adjuvanticity. Peptides will be selected among the most immunogenic sequences that are retained in rat, mouse and human HER2 (universal peptides), able to bolster HER2-specific CD4+ and CD8+ responses.

Experimental design

The efficacy of vaccine prototypes will be assessed and compared with clinically relevant adjuvants/peptides comparators. The magnitude and longevity of the humoral and cellular Ag-specific immune responses will be assessed in both the preventive, preneoplastic and therapeutic settings, using different mouse strains and transgenic models. The best performing prototypes will be also investigated in combination therapies with clinically-relevant options. To pinpoint the mechanism of action responsible for HA adjuvanticity, different mouse strains and transgenic mouse models will be employed, together with in vivo depletion of immune cell subsets. Quantitative biodistribution of HA-vaccines and the involvement of LYVE-1 receptor will be assessed by radioimaging, while the major immune cell players involved will be deciphered both in vitro and in vivo using multiple techniques such as quantitative multispectral digital pathology microscopy, gene expression analysis, confocal microscopy, and flow cytometry.

Expected results

We expect to characterize and validate at least one HA-based vaccine prototype that is capable of strongly interfering with the growth of HER2+ BC. The concomitant thorough assessment of the mechanism of action and toxicology will support the rapid transfer from the bench to bedside, and the scale up to clinical trials.

Impact on cancer

Cancer is a major public health problem worldwide, and the recent COVID-19 pandemic demonstrated how its management can be hampered in situations of global public health emergencies. The peerless versatility of our HA-based vaccine technology might have an extremely significant social impact at different levels, as it would offer new solutions for both preventing the development of different hereditary neoplasms and providing new effective weapons for the therapy of existing neoplasms and relapses of disease.

Matteo Cereda **Bridge Grant - 2023**

Università degli Studi di Milano

Targeting mRNA processing defects in adult and pediatric cancer

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Background

RNA therapeutics have revolutionized our approach to disease therapy, including cancer. Targeting aberrant mRNA isoforms with a driver role in cancer offers the opportunity to increase the list of therapeutic options for patients. So far, conventional therapeutic approaches showed limited success in treating patients with aggressive, heterogeneous, and resistant tumors.

Hypothesis

Our working hypothesis, sustained by our published and preliminary results, is that aberrant mRNA processing, particularly alternative splicing, holds the potential to increase the number of actionable targets. For this reason, it represents an interesting ground for developing RNA-based therapeutic strategies.

Aims

Our goal is to identify and target mRNA defects in prostate cancer, pediatric bone sarcomas, triple-negative breast cancer, and KRAS-mutated cancers. We intend to provide a list of tested splicing switching oligonucleotides (SSOs) able to target oncogenic mRNA isoforms and block tumor progression, and (2) systematically assess the causative mechanisms of defective mRNA processing across and within these cancer types. Our final goal is to increase the short list of therapeutic targets for the clinical management of pediatric and adult patients suffering from these rare or aggressive cancers.

Experimental design

Starting from our published and preliminary data, we will analyze short- and long-read sequencing data to identify novel actionable mRNA-processing-related regulators and targets in rare and aggressive tumors. We will design SSOs for candidate oncogenic events and assess their actionability in preclinical models of prostate, bone sarcoma, and KRAS-mutated cancers.

Expected results

We expect to: (1) identify mRNA-processing-related regulators and aberrant mRNA isoforms driving tumor progression and/or resistance to treatment; (2) characterize the causative mechanisms of aberrant mRNA processing; (3) uncover novel RNA-based therapeutic targets useful for clinical management of these patients; (4) provide experimentally validated SSOs blocking oncogenic targets.

Impact on cancer

Assessing mRNA processing deregulation in cancer will provide the scientific community with a list of additional oncogenic targets, particularly useful in tumors where other somatic variations might be absent. An accurate evaluation of the actionability of these RNA targets will be instrumental for the delivery of future personalized therapeutic treatments.

Federica Cossu

Consiglio Nazionale delle Ricerche

Targeting BIR-mediated onco-PPIs: rational design of NF-κB modulators

Background

The over-expression of Inhibitors of apoptosis proteins (IAPs) family enhances cell survival and resistance to chemotherapics. IAPs-mediated complexes ubiquitylate substrates thus regulating NF-κB pathway. Type I BIR (Baculovirus IAP repeat) domains of IAPs are pivotal for the assembly of such complexes. IAPs' Type-II BIRs directly interact with caspases to inhibit cell-death, or with SmacDIABLO for apoptosis restoration. Type II BIRs-directed therapies, Smac-mimetics (SMs), relieve caspases from inhibition by X-linked IAP and induce cIAPs (cellular IAP1 and 2) auto-ubiquitination and degradation. BIR-mediated Protein-Protein Interactions (PPIs) are validated onco-targets.

Hypothesis

IAPs-directed therapies are designed targeting pockets or hotspots on isolated, globularly structured BIR domains. However, BIRs relative positioning within the entire IAP molecule is the key for various pro-survival roles. Besides our, few groups managed to isolate full-length IAPs obtaining the yields required for structural studies. SMs, now in advanced phases of clinical trials, produce divergent effects, as observed in SMs-resistant cancer cell lines, where cIAP2 is upregulated promoting cell survival. Information about the structural details of full length IAPs is necessary. In fact, SMs were hypothesized to induce huge IAPs re-arrangements, but it has not been fully demonstrated yet. Furthermore, we identified compounds binding BIR1-mediated PPI surfaces, showing disruption of IAPs-containing complexes and IAPs-dependent cell-toxicity.

Aims

The project targets BIR-mediated onco PPIs to (i) develop/improve IAPs-targeting therapies and (ii) unravel the molecular determinants of the action of IAPs or IAPs-inhibitors. To this purpose, we aim at identifying FL-IAPs constructs suitable for structural analysis, also in the presence of partners and ligands. Furthermore, structure-driven and bio-chemical/-physical approaches will allow the selection of modular IAPs-selective molecules, ultimately tuning NF-κB.

Experimental design

We propose to expand the chemical space around the lead Cmp2, generating a new class of potential cancer therapics. Furthermore, we will consider the synthesis of hybrid compounds, coniugating anti-BIR1 compounds with SMs. We plan to investigate the triggered cellular pathways on a large panel of tumoral cell lines and characterize the biophysical properties underlying their action. Particular attention will be paid to cells described as resistant to IAPs-antagonists or other chemotherapeutic agents after IAPs activation. The structure-based approach will reveal details on protein-ligands interactions and consequent conformational changes of IAPs, providing the rationale for drug lead optimization. Furthermore, the virtual screening of compounds libraries against the new IAP structural hotspots will expand the number of chemicals to be included in the development of new anti-cancer candidates.

Expected results

We expect to characterize a library of novel putative anti-cancer molecules in vitro, and to propose 2-3 candidates for in vivo tests. We expect to obtain at least one structure for each FL-IAP homologue, with a partner and/or a ligand. A deeper understanding of the action of IAPs at the molecular level and the use of high-resolution techniques represent steps forward the development of optimised treatments.

Impact on cancer

The modulation of pro-survival complexes regulating the NF-κB pathway, as the ones mediated by IAPs, can be the strategy to overcome cases of resistance to current IAPs-targeting chemotherapics, to better define IAPs roles/functioning and find accurate/selective therapies.

Stefano Crippa **Next Gen Clinician Scientist - 2024**

Università Vita-Salute San Raffaele

A novel integrated approach to risk stratification of intraductal papillary mucinous neoplasms of the pancreas

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Background

IPMNs are frequently detected, precancerous lesions of the pancreas that can develop into PDAC, a deadly cancer. We need better ways to identify IPMNs at risk of progression to PDAC to guide clinical decisions and improve patient outcomes.

Hypothesis

A multi-omics biomarker panel for malignancy detection in peripheral blood (PB) and/or IPMN cyst fluid, and artificial intelligence (AI)/machine learning (ML) models for IPMN progression can enable earlier detection of malignancy in IPMNs for personalized management and treatment.

Aims

'-AIM 1: To develop (WP1) and validate (WP2) a multi-omics biomarker panel for malignancy (presence of high-grade dysplasia or invasive cancer) detection in IPMNs patients.
-AIM 2: To develop (WP3) and validate (WP4) AI/ML models to enhance the stratification of IPMNs at diagnosis into high-risk, low-risk, or no-risk categories for progression to cancer.

Experimental design

The experiment will be conducted in four workpackages (WP):
-WP1. We will develop a multi-omics biomarker panel to detect malignancy in IPMNs. To do this, we will collect a wide range of data from PB and IPMN cyst fluid samples from 310 patients with resected IPMN. The data will include clinical/exposome data, radiomics, somatic/germline mutations, and metabolic/proteomic markers. We will then use nonparametric epidemiologic methods and AI/ML models to identify a robust biomarker panel that can accurately identify malignant IPMNs.
-WP2. Once we have developed a multi-omics biomarker panel, we will test its performance in a group of IPMN patients who are scheduled to have surgery or surveillance after endoscopic ultrasound. This will allow us to assess the sensitivity, specificity, and accuracy of the biomarker panel. Germline variants strongly associated with malignancy will be validated in PANDoRA consortium.
-WP3. We will develop AI/ML models to predict the risk of IPMN progression to cancer. We will collect clinico-radiological and follow-up data from a large cohort of patients with IPMN under surveillance (n=1500) and of resected IPMNs (n=550). We will use this data to train AI/ML models to identify patterns associated with a higher risk of progression to cancer.
-WP4.We will test the performance of our AI/ML models on a large group of IPMN patients with long-term follow-up data from different international high-volume centers. We will compare the models' predictions to the patients' actual outcomes to assess their accuracy.

Expected results

This study will develop and validate a multi-omics biomarker panel for the detection of IPMNs at risk of being or becoming malignant and AI/ML models for IPMN progression stratification.

Impact on cancer

'- Early detection of IPMN at risk of progression to PDAC has the potential to prevent the development of advanced pancreatic cancer thus improving survival rates.

-AI/ML-based risk stratification models will facilitate personalised treatment strategies for IPMNs, with better resources allocation and minimising unnecessary interventions for low-risk patients.

-The analysis of multi-omics data associated with IPMN progression to malignancy will increase our understanding of pancreatic carcinogenesis, enabling the identification of novel potential therapeutic targets.

Francesco Cucco **MFAG - 2023**

Consiglio Nazionale delle Ricerche

FUNCTIONAL CHARACTERIZATION AND DIAGNOSTIC/PROGNOSTIC IMPACT OF MYC POINT MUTATIONS IN DLBCL

Background

Diffuse large B-cell lymphoma (DLBCL) is the most common haematological malignancy and accounts for more than 80% of high-grade lymphomas. MYC translocation (MYC-R) occurs in 10% to 15% of DLBCL. In DLBCL, MYC-R is associated with different partners and those with an immunoglobulin (IG) gene confer inferior clinical outcome. The reason for this is unclear. We identified highly frequent MYC point mutations in DLBCL cases with a MYC/IG genes translocation (~65%). The nature of these mutations is uncertain. Most likely they are caused by the aberrant somatic hypermutationmachinery (aSHM) activity, but their functional impact on MYC and cells biology is still largely unknown.

Hypothesis

MYC is frequently deregulated in cancer, mostly due to amplification and translocation events. MYC point mutations have been found in a variety of solid and non-solid cancers, but only few of them have been functionally tested so far. These mutations could have a previously underestimated impact on cancer evolution, particularly in DLBCL, where they could act in synergy with MYC/IG translocation. Therefore, these mutations can be potentially used as prognostic/diagnostic biomarkers and drive treatment decision in the clinic.

Aims

•To identify and expand the spectrum of pathogenic mutations that enhance MYC protein stability and trigger cell transformation.

•To investigate all relevant MYC mutations for further characterization in vitro and in vivo.

•To comprehensively assess the prognostic value of MYC translocation/mutation in a large cohort of DLBCL MYC-R cases.

•To evaluate if MYC mutations can be used for the early detection of the high-risk DLBCL with MYC/IG gene translocation at the time of diagnosis

Experimental design

In the first part of the present proposal, we will perform in vitro and in vivo experiments employing different cell lines and animal models in order to functionally characterize the MYC point mutations previously identified in DLBCL MYC-R cases. The second part of the investigation will be focused on the molecular characterization of DLBCL samples. This will be achieved by using fluorescent in situ hybridization (FISH) and a combination of sequencing approaches like Digital Droplet PCR (ddPCR) and Next-Generation Sequencing (NGS).

Expected results

By the end of the project, we anticipate to have a full picture of the impact of the investigated MYC point

mutations in terms of enhancing MYC protein stability, transformation capacity and tumorigenesis. Moreover, the correlation of the MYC mutation status with different molecular and clinical data in a large cohort of DLBCL MYC-R cases will build up an integrated pathway for the prognostic stratification of these cases that might be used in a routine clinical setting.

Impact on cancer

This work will shed light on the role of MYC point mutations in B cell malignancies. It will also elucidate the translational potential of the relevant MYC point mutations, defining new prognostic markers for high risk DLBCL patients, improving diagnosis, and establishing new targeted therapies. On a larger scale, this work also has the potential to be extended to other malignancies sharing similar genetic features.

Matteo Claudio Da Vià **MFAG - 2024**

Fondazione I.R.C.C.S. Ca' Granda Ospedale Maggiore Policlinico

Tracking the phenotypic plasticity of Multiple Myeloma at diagnosis and after treatment by single-cell genotypic methods

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Background

Multiple Myeloma (MM) is a plasma cell (PC) disorder, characterized by a huge genomic heterogeneity which fuels disease evolution from asymptomatic stages to symptomatic ones until therapy refractoriness. This heterogeneity could be paralleled also by a phenotypic cell heterogeneity able to shape a more complex clonal MM cell reservoir responsible for the disease onset, evolution and therapy resistance.

Hypothesis

The leading postulate of this proposal is that a MM cell reservoir exists in the BM, characterized by phenotypic heterogeneity but sharing the same clonal features of the MM PCs, already present at the disease onset and able to sustain clonal evolution and refractoriness at the minimal residual disease (MRD) level.

Aims

AIM1: Genomic, transcriptomic and phenotypic dissection of MM clonal cells reservoir in asymptomatic and symptomatic MM patients at diagnosis.

AIM2: Single molecule tracking of MM cell reservoir during spontaneous disease evolution and after treatment.

AIM3: Identification of specific vulnerabilities at MRD in genotypically defined single MM cells.

AIM4: Development and establishment of modeling of MRD positive cell in 3D silk bioink model of MM-BM niche.

Experimental design

I aim to prospectively enroll 15 smoldering-MMs and 20 newly-diagnosed (ND) transplant eligible MM patients. At diagnosis, PCs (CD138+/CD38+), B cells (CD19+/CD138-/CD38-) and plasmablasts (CD19+/CD38+/CD138-) will be sorted by FACS and undergo to whole genome and targeted deep sequencing analysis. An aliquot of BM mononuclear cells will be sequenced by single-cell RNAseq with V(D)J and CiTEseq analysis. This workflow will be applied at disease progression and at MRD to identify genotypically-defined MM cells that are phenotypically aberrant. Patients will be followed-up in the PB through the innovative ultra-sensitive Rolling Circle Amplification technology to track patient-specific mutations, translocations and the V(D)J rearrangements, in collaboration with Rarity biosciences. Finally, I will model residual MRD-positive cells in a 3D printed ex-vivo BM model to model pharmacological vulnerabilities and test new generation immunotherapies such as bi- and tri-specific antibodies in strict collaboration with Alessandra Balduini's lab and Ichonos-Glenmark biotech.

Expected results

1) the depiction of the genomic architecture of phenotypically different but hierarchically-related cells in

asymptomatic patients, deciphering the cellular origin of primordial events in PCs dyscrasias;
2) the definition of clonal dynamics on the whole clonal cell population, deciphering the cell and molecular composition at different timepoints;
3) the detection of specific gene signature able to predict MRD persistence after therapy;
4) the implementation of highly sophisticated, cutting-edge and clinical grade technologies for the pheno-genotypically redefinition of whole MM clonal cell reservoir at diagnosis and at MRD and technics to overcome therapy resistance.

Impact on cancer

The knowledge of cancer primordial founding events and the early interception of tiny resistant clones after therapy are fundamental aims for early cancer detection and deep eradication of residual tumoral cells. This proposal combines cutting-edge technologies and an innovative framework of analysis aiming to define phenotypically and molecularly early driver events in different clonal population and to track and eradicate them during the early phases of the tumor.

Jessica Dal Col

Università degli Studi di Salerno

Role of RNA-binding protein HuR in target therapy resistance and immune evasion in EGFR-driven lung cancers

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Background

Small molecule target therapy with tyrosin kinase inhibitors (TKIs) is the first-line therapy recommended for patients with epidermal growth factor receptor (EGFR)-driven non-small cell lung cancer (NSCLC). Despite EGFR-TKI clinical efficacy, acquired resistance invariably develops. Alterations of cell cycle and establishment of immunosuppressive tumor microenvironment (TME) contribute to resistant cells phenotype. Insights and approaches to overcome and/or prevent resistance are critical unmet needs, especially regarding EGFR-independent mechanisms of resistance often shared among the different EGFR-TKIs.

The RNA-binding protein Hu antigen R (HuR) participates in cancer pathogenesis by posttranscriptional regulation of stability, cellular compartmentalization, and translation of multiple transcripts involved in all hallmarks of neoplastic transformation, immune escape and treatment resistance. Targeting HuR is intensely studied for cancer therapeutics. HuR role in EGFR-independent mechanisms of acquired resistance in NSCLC is poorly defined, as its potential targeting.

Hypothesis

Our published and preliminary results and recent literature strongly indicate HuR as important determinant in mechanisms driving both proliferation and immune escape in EGFR-driven NSCLC following TKI resistance acquisition. We hypothesize HuR involvement in sustaining tumor cells proliferation and survival, and promotion of immunosuppressive TME through sustained production of cytokines/chemokines and expression of immune-checkpoint molecules through multiple posttranscriptional mechanisms influencing its targets' mRNA stability, translation, or proteins membrane localization.

Aims

We will investigate the role of HuR in promoting tumor growth in EGFR-TKI- resistant NSCLC models by its influence on tumor cell proliferation, survival, migration/invasion, and immune escape. To address this goal, we propose to evaluate (1) HuR impact in mediating proliferation and survival in EGFR-TKI resistant cells and (2) in immune evasion of EGFR-TKI resistant cells through multiple components of immunesurvaillance; (3) HuR expression in patient-derived tissue specimens and cell line models, in circulating tumor cells (CTCs) and HuR-regulated, EGFR-TKI-dependent effects on circulating immune parameters in NSCLC patients.

Experimental design

HuR-dependent functions will be tested in EGFR-TKI resistant NSCLC cells with physiological/exogenously altered HuR levels (stably/conditional ablation by CRISPR-Cas9/doxycycline-inducible shRNA, retrovirally induced overexpression, pharmacological inhibition). Specific HuR-mRNA interactions will be investigated by Clip-Seq/RNA-Seq parallel analysis and validated by immunoprecipitation/biotin pulldown; specific assays will test mRNA stability and translation; readouts of proliferation/survival, immune escape mechanisms (secretome-driven immunosuppressive TME, immune-checkpoint regulators expression, neutrophils

chemotaxis/functions, cell-mediated cytotoxicity) will be measured in vitro and, partially, in humanized mouse models. HuR and targets expression in patient-derived tissue specimens and CTCs will be characterized by single-cell transcriptome analysis and immunofluorescence or immunohistochemistry, HuR-related circulating immune components by flow cytometry, multiparametric/single ELISA.

Expected results

We expect to identify HuR-regulated potentially therapeutically targetable molecular determinants of tumor growth and immune evasion involved in EGFR-TKI resistance. Results may help devise strategies addressing specific HuR:mRNA target interactions possibly relevant for acquired therapy resistance or for turning "cold" tumors into "hot".

Impact on cancer

Identification of HuR-driven mechanisms in EGFR-TKI failure would support studying novel strategies to maintain in these patients a much-needed response to other existing anti-cancer therapies, including immunotherapy. Given HuR role in other solid tumors, the study could identify therapy resistance mechanisms of broader mechanistic and clinical relevance.

Sara De Biasi **MFAG - 2023**

Università degli Studi di Modena e Reggio Emilia

Unravelling the role of mucosal associated invariant T cells in non-small cell lung cancer

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Background

Circulating mucosal associated invariant T (MAIT) cells are an innate-like pro-inflammatory and cytotoxic population of effector memory T cells and can represent up to 10% of peripheral CD8+ T cells. They recognize microbial proteins presented by non-polymorphic MHC class I related-molecule (MR1), display homing properties and are characterized by the expression of semi-invariant Va7.2 T cell receptor (TCR) combined with high level of the inhibitory receptor CD161. MAIT cells are deeply involved in orchestrating the immune response in the mucosae. Controversial data exist regarding the role of MAIT cells in cancer. We recently showed that circulating MAIT cells predict response to anti-PD1 therapy in metastatic melanoma patients and they reside in the tumor microenvironment (TME), at higher percentage in responding patients. MAIT cells are enriched also in tumor lesions of non-small cell lung cancer (NSCLC) patients, but scanty data exist on cell-cell interactions and mechanism(s) of function of these cells in the TME. Recently, a rare population of MAIT cells has been depicted as capable to respond to tumor cells of different tissue origin.

Hypothesis

Based on recent preliminary and unpublished data, MAIT cells play a cancer-specific cytotoxic role in NSCLC and show potential predictive value for recurrence. The main aim of the study is to better depict the identity of MAIT cells, to identify their function and role within the TME of resectable NSCLC, and to unravel the mechanisms and interactions at the basis of their function.

Aims

We plan to profile and modulate this population in NSCLC, and in particular to: 1. provide new information on the structure, organization, and relationship of the immune and tumor synapses; 2. understand if they are in fact main actors of the immune response; 3. identify the rationale and develop possible tools for modulating their action.

Experimental design

In this five-year project, on a total of 80 patients with resectable NSCLC, we will: 1) broadly characterize MAIT cells in the TME and their immune synapses, by investigating their spatial localization and interaction, decipher their phenotypic, functional and metabolic profiles and correlate immunological data with clinical outcome; 2) evaluate MAIT cells response to tumor associated antigen (TAA); 3) assess a rationale for MAIT function modulation by interfering with the CD161-LTT1 axis in vitro, decipher an anti-cancer immune response of MAIT cells in lung cancer tumor-spheroid-MAIT-cell coculture system and reprogram and redifferentiate MAIT cells (reMAIT).

Expected results

We will expect to clarify the role of MAIT cells, finding their main interactors in TME and quantify their anti-

cancer response. We expect to pinpoint new regulating mechanisms of cytotoxicity exerted by MAIT cells and to identify new MAIT-related features associated with clinical outcome of NSCLC.

Impact on cancer

The 5-year survival of NSCLC patients is low. For this reason, given that MAIT cells are cytotoxic, reluctant to cause graft-versus-host disease, are cancer-drug resistant (they express multidrug efflux pump such as CD243) and reinforce expansion and the effector functions of NK cells in tumor, the advent of induced pluripotent stem-cell-derived MAIT cells (reMAIT cells) will make it possible to harness these cells for immune cell therapy.

Antonio De Leo **MFAG - 2021**

Alma Mater Studiorum Università di Bologna

Integrated molecular analysis of endometrial carcinoma: translation of biomarker profiles into the clinical practice.

Background

Endometrial cancer (EC) is the most common gynecological cancer and 15-20% of EC patients have an aggressive clinical course. The Cancer Genome Atlas (TCGA) project identified four distinct prognostic EC subgroups based on molecular alterations: (i) POLE mutated tumors - excellent prognosis; (ii) MisMatch Repair deficient (MMRd) tumors - intermediate prognosis; (iii) p53 abnormal/mutated tumors (p53abn) - poor prognosis; (iv) No Specific Molecular Profile (NSMP) tumors - intermediate prognosis. Although the four molecular groups appear to be different in prognosis, the NSMP subgroup (the vast majority of EC cases) represents a heterogeneous group of tumors with variable molecular alterations and divergent clinical outcomes. Translation of TCGA molecular groups into clinical practice is emerging as a challenge. In addition, an interesting aspect in EC characterization is represented by the impact of the tumor microenvironment on tumor growth, progression, and responses to therapies, especially immunotherapies.

Hypothesis

Integrated study through multidisciplinary and multimodal approaches is essential to deepen the understanding of the clinical and biological complexity of endometrial cancer, refining the predictive-prognostic stratification of patients and providing the rationale for individualized forms of treatment.

Aims

This project aims:

- To integrate novel immuno-molecular markers with TCGA molecular subgroups to investigate tumor heterogeneity, improve risk stratification, and identify potential target pathways;
- To explore the predictive-prognostic impact of the tumor immune microenvironment;
- To integrate DNA and RNA profiles to obtain robust biomarkers relevant to risk stratification;
- To investigate the role of liquid biopsy as a non-invasive alternative to improve diagnosis and early detection of recurrence.

Experimental design

'- Enrollment of 300 EC patients
- Collection of comprehensive clinical data and tumor tissue samples
- Immuno-molecular subtyping of EC by comprehensive clinicopathological, immunohistochemical and Next Generation Sequencing (NGS) analysis
- Characterization of EC immune microenvironment with gene expression profiles and immunohistochemistry on tumor tissues
- RNA sequencing and miRNA expression profiles on tumor tissues
- Molecular analysis of liquid biopsies (uterine aspirates and blood) to detect molecular alterations, circulating tumor DNA, and circulating miRNAs.

Expected results

'- Identification of molecular alterations useful for risk stratification and targeted therapy;

- Identification of an immune gene signature for predicting the prognosis and guiding immunotherapy treatment;

- Definition of an immunoscore feasible and useful in clinical practice;

- Identification of differentially expressed genes in molecular EC subgroups correlated to prognosis and clinically targetable pathways;

- Definition of miRNA expression profiles related to different molecular subgroups and to prognosis;

- Development of an integrated liquid biopsy in EC patients to improve diagnosis, patient surveillance and early detection of recurrence.

Impact on cancer

The project contributes to a more comprehensive understanding of the biological, pathological, molecular and clinical features of endometrial cancer through a multi-method and multidisciplinary approach. This project, thanks to the synergy of different methodologies, will result in a deeper exploration of intra- and inter-tumor heterogeneity and it will define informative prognostic and predictive factors applicable in clinical practice. The results of the project are expected to have an impact in a more accurate patient management and in the development of personalized treatments.

Donatella Delle Cave **MFAG - 2024**

Consiglio Nazionale delle Ricerche

Mitigating pancreatic cancer aggressiveness by targeting epithelial matrisome components

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Background

Pancreatic ductal adenocarcinoma (PDAC) remains a devastating disease with dismal prognosis. Despite significant progress in comprehending the fundamental biology of PDAC, these advancements haven't translated into substantial improvements for patients. Tumor progression, metastasis, and chemoresistance are heavily influenced by the dense desmoplastic stroma, particularly the matrisome, an intricate network of extracellular matrix (ECM) proteins and associated factors. The matrisome, rich in collagen and laminins, plays a critical role in fibrosis development. However, a comprehensive understanding of its composition in PDAC is lacking.

Hypothesis

While stromal cells are the primary source of the matrisome, emerging evidence suggests that epithelial cancer cells also contribute to its secretion, potentially promoting tumor fibrosis and chemoresistance. The impact of collagen and laminins derived from tumor cells on PDAC growth, progression, and ECM stiffness remains largely underestimated, with no targeted therapeutic strategies currently explored. Deciphering how alterations in matrisome secretion influence PDAC aggressiveness is crucial for developing more effective treatments.

Aims

The main goal of the project is to decipher the "matrisome code" to target PDAC tumorigenicity. Our research seeks to challenge conventional understanding by emphasizing how specific tumor populations actively alter the ECM within the pancreatic tumor microenvironment. Specifically, we will: 1) investigate the role of epithelial cancer cells in tumor fibrosis by identifying the specific components of the matrisome they secrete, 2) characterize the metabolic profiles of the primary matrisome-secreting cells, 3) inhibit matrisome secretion to enhance the effectiveness of current chemotherapy in suppressing tumor growth and metastasis, 4) validate these findings in preclinical studies to improve patient prognosis.

Experimental design

We will employ an integrative approach, combining cell biology techniques, genetically engineered mouse models and omics analyses. PDAC tumors will undergo mass spectrometry analysis to define the distinct proteomic signatures of epithelial and stromal cells. CRISPR/Cas9 technology will be utilized to identify genes and cell populations significantly contributing to the tumor matrisome. Label-free methodology will be applied to profile the metabolic features of the main matrisome-secreting cells. Lastly, a preclinical mouse model that recapitulates the complex PDAC tumor microenvironment will be used to assess the therapeutic potential of combining chemotherapy with novel antifibrotic drugs in reducing tumor growth.

Expected results

This research tackles PDAC from a novel perspective, focusing on the matrisome composition. Our discoveries will elucidate the impact of matrisome origin on PDAC aggressiveness and metastasis, identifying metabolic alterations that lead to increased ECM secretion from tumor cells. We will explore therapeutic interventions to mitigate the tumorigenic potential of matrisome-secreting cells by targeting their metabolic pathways.

Impact on cancer

Our findings will establish novel pathophysiological roles and functions for matrisome components, and will enable us to customize therapeutic strategies based on the particular cell types driving PDAC's aggressive behavior. The recognition and validation of the distinct matrisome signatures will facilitate patient stratification and predict treatment response. Importantly, the findings from this project could potentially be applicable to other types of tumors characterized by extensive fibrosis.

Marta Di Martile **MFAG - 2024**

Istituti Fisioterapici Ospitalieri (IFO)

Exploring the role of Bcl-2 family in the immune surveillance of melanoma: from mechanisms to therapeutic perspectives

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Background

The rational development of new therapeutic combinations addressing the resistance to immune checkpoint blockade (ICB) therapy is a clinical need to improve the management of metastatic melanoma patients. Several pieces of evidence identified that strategies increasing the antigen presentation and interfering with the detrimental crosstalk between the tumor cells and those of the surrounding tumor immune microenvironment (TIME), significantly improve the ICB response.

BH3 mimetics, small molecules able to interfere with the function of Bcl-2 family anti-apoptotic proteins, have gained excitement with the recent success in hematological malignancies. Their antitumor efficacy, when administered alone or in combination with chemo- or targeted- therapy, has been characterized in different preclinical solid models, and clinical trials are evaluating the safety and efficacy of BH3 mimetics in combination regimens in solid tumors, including melanoma. Our preliminary results indicate that Bcl-2 inhibition may increase the expression and the membrane exposure of MHC I and some molecules belonging to the antigen-presenting machinery, and could potentiate the ICB treatment.

Hypothesis

The anti-apoptotic members of the Bcl-2 family, particularly Bcl-2, could elude the immune surveillance of melanoma by acting on both tumor cells and those of the TIME. Bcl-2 inhibition may increase melanoma immunogenicity, thus potentiating the efficacy of ICB therapy.

Aims

This project aims to investigate whether Bcl-2 inhibition can: i) increase the melanoma immunogenicity by regulating the MHC I molecules and the antigen diversity, and modulating the extracellular vesicles (EVs) release and content; ii) affect the tumor-infiltrating immune cell subpopulations and their activation; iii) potentiate the immunotherapy treatment in ICB-resistant melanoma models.

Experimental design

A panel of established murine and human melanoma cell lines, and patient-derived metastatic melanoma cells will be used for the in vitro experiments. The ability of Bcl-2 inhibition (through genetic or pharmacological approaches) to regulate the expression levels or the functionality of MHC I molecules or those of the antigen-presenting machinery will be investigated together with the involved molecular mechanisms. Proteomic analysis will be used to identify the ability of Bcl-2 inhibition to regulate the peptide repertoire generated by melanoma cells. EVs isolated by conditioned media from melanoma cells with different levels of Bcl-2 obtained through genetic or pharmacological approaches and from plasma samples of tumor-bearing mice treated with BH3 mimetics will be analyzed to evaluate the ability of Bcl-2 inhibition to modulate EVs assembly, release, and content. In vivo, low or highly immunogenic murine melanoma models will be used to analyze the ability of Bcl-2 inhibitors (ABT-199, ABT-263) to modulate the recruitment

and activation of immune cell subsets, potentiate the efficacy of ICB therapy and overcome ICB resistance. Finally, the antitumor efficacy of the combinatorial regimen will be evaluated by using patient-derived organotypic cultures from metastatic melanoma patients.

Expected results

We expect to develop new Bcl-2-based therapeutic strategies to address the ICB resistance and to identify novel functions of the Bcl-2 family in mediating the crosstalk between melanoma cells and those of the TIME.

Impact on cancer

Targeting the Bcl-2 family members may provide an alternative way to increase melanoma immunogenicity and improve the response to ICB therapy.

Orsola Di Martino **Start-Up - 2024**

Università degli Studi di Parma

Define the role of DDIT4 in Acute Myeloid Leukemia progression and how it affects chemotherapy-response mechanisms

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Background

Acute Myeloid Leukemia (AML) is the deadliest form of leukemia both in adults and children. This dismal survival rate depends mostly on the high rate of chemoresistance. The standard treatment for AML consists of intensive induction chemotherapy, often cytarabine (AraC) with anthracyclines and bone marrow transplantation. The combination of venetoclax and azacitidine has also emerged as a therapy for older patients or those unfit for intensive chemotherapy. However, recent studies indicate that the majority of patients ultimately develop resistance to therapy as well. Despite these high rates of therapy failures, the molecular changes that confer chemotherapy resistance are still unknown.

Hypothesis

Chemotherapy-resistant AML cells display significant metabolic plasticity compared to de novo leukemia. In search of downstream effectors of the IRE1a-XBP1s pathway that is critical to AML pathogenesis and is activated by the first-line chemotherapies Ara-C and Doxorubicin, I have identified DDIT4 as a potential mediator of chemotherapy resistance. High DDIT4 levels are associated with worse outcomes in AML, breast cancer, glioblastoma, colon skin, and lung cancer. DDIT4 expression is elevated in AML subtypes that are generally associated with chemotherapy resistance, such as in MLL-AML, FLT3-ITD NK-AML and complex karyotype (CK-) compared to healthy BM cells. First, I hypothesize that AML cells rely on DDIT4 to support AML cell survival and resistance to standard of care AML therapies. Second, I hypothesize that DDIT4 localizes to mitochondria and interacts with TCA enzymes and ETC components to regulate OXPHOS and ATP production and prevent premature mitochondrial exhaustion in AML cells.

Aims

To define the role of DDIT4 in AML and how it affects chemotherapy resistance mechanism, the proposal is articulated in two main Aims:

Aim1.Determine how DDIT4 modulation impacts AML pathogenesis and chemotherapy response.

Aim2: Our preliminary studies show that DDIT4 interacts with key metabolic proteins , for this reason, I'll investigate the role of DDIT4 in mitochondria metabolism.

Experimental design

To address the scientific questions that arise from Aim 1: I will assess the role of DDIT4 in the progression and chemoresistance of aggressive AML genetic subtypes and how DDIT4 deletion impacts healthy hematopoiesis. Then, I will test the therapeutic potential of pharmacologically targeting the IRE1a-DDIT4 pathway in patient-derived leukemia cells.

From Aim 2: I will identify which mitochondrial protein have a direct interaction with DDIT4. Then, I will detect which DDIT4-interacting proteins are critical for chemoresistance in AML. Lastly, I will investigate how DDIT4 expression influences mitochondria fuel source utilization.

Expected results

I expect that the combination of DDIT4 inhibition and standard chemotherapy will substantially enhance the efficacy of chemotherapy in resistant AML subtypes. Moreover, I predict that DDIT4 inhibition will impede AML cells' mitochondria from switching metabolism and support chemoresistance mechanisms.

Impact on cancer

If successful, these studies will help to define how DDIT4 regulates mitochondrial metabolism to support AML cell growth and chemoresistance and, as a result, may have significant therapeutic benefits in AML as well as the many other cancers affected by dysregulation of these processes.

Rosa Di Micco

Next Gen Clinician Scientist - 2023

Università Vita-Salute San Raffaele

Tailoring treatment of luminal A and lobular breast cancer with 18F FES-PET/MRI

Background

Breast cancer (BC) management has evolved towards a tailored treatment mainly based on tumor biology and patient's characteristics. Staging at diagnosis is crucial to build up the most appropriate therapy. However, imaging performance is not equivalent in all subtypes. In particular, staging of luminal A BC(LumA) and lobular BC(Lob) remains complex. On one side, LumA defined as being estrogen receptor(ER)- and progesterone receptor-positive, well-differentiated, Her2-negative with a low proliferation index (< 20%) has worse sensitivity on axillary US, reduced MRI enhancement and low FDG-avidity on PET. On the other side, Lob presents a peculiar growth pattern that makes MRI less sensitive on axillary and systemic staging and FDG-avidity lower. Despite the relatively favorable prognosis, nodal metastases and recurrence are possible and, as LumA and Lob account for >50% of all BCs, their absolute values are higher compared with other subtypes with a concrete risk of disease underestimation and undertreatment. Genomic testing showed that a high genomic risk of recurrence could be hidden even behind low clinical risk tumors. To date, obtaining an initial reliable staging of LumA and Lob in order to plan the most appropriate treatment pathway and to minimize the recurrence risk still represents a partially unmet clinical need. In San Raffaele Hospital, two ongoing studies on FDG-PET/MRI in BC staging showed that its sensitivity decreases in these BC types.

Hypothesis

Our hypothesis is that combining the advantages of hybrid PET/MRI and the high sensitivity/specificity of 16-alpha-18F-fluoro-17-beta-estradiol(FES), a radiolabeled form of estrogen binding to functionally active ER, we could obtain a reliable, non-invasive, operator-independent, one-stage imaging method for staging LumA and ER-positive Lob.

Aims

Aim 1: Evaluating the performance of FES PET/MRI in axillary staging compared with axillary surgery.
Aim 2: Evaluating potential correlations between changes in FES uptake and changes in proliferation index after three weeks of endocrine therapy before surgery.
Aim 3: Evaluating the performance of FES PET/MRI in systemic staging of patients undergoing systemic therapy in comparison with standard imaging.
Additionally, biological determinants of tumor heterogeneity will be investigated.

Experimental design

This is a prospective cohort study where patients with LumA and ER-positive Lob will be enrolled in four cohorts undergoing: primary surgery; induction endocrine therapy; neoadjuvant chemotherapy; systemic therapy for metastatic disease. For the purpose of the study an additional FES PET/MRI exam will be performed at baseline for local and systemic staging and a second exam after systemic therapy. Correlations between FES PET/MRI parameters and pathology, gene expression, CTCs, and FDG PET parameters, when available, will be investigated.

Expected results

We expect that results from this prospective trial will allow a personalized BC staging in LumA and Lob providing evidence of FES PET/MRI performance in different settings: early and advanced BC, response prediction and monitoring.

Impact on cancer

Studying the performance of FES PET/MRI in selected BC cohorts will have an important impact on the stratification of BC patients, allowing a customization of surgery, radiotherapy and systemic therapy, and to potentially improve patient prognosis. Furthermore, using a non-invasive molecular and functional imaging could positively affect healthcare system reducing direct and indirect BC-related costs.

Diego Dibitetto **Start-Up - 2023**

Istituto di Ricerche Farmacologiche "Mario Negri" I.R.C.C.S.

A Proteo-Genomic Approach to Study DNA-PK's Function in PARP Inhibitor Resistance

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Background

DNA-PKcs is a kinase with key roles in DNA double-strand break repair. Recently, additional roles for DNA-PKcs have been described in RNA metabolism and activation of interferon response, but the underlying mechanisms have not been elucidated. DNA-PKcs is frequently over-expressed in advanced cancers and, for this reason, DNA-PKcs inhibitors have been developed and are currently being tested as adjuvant agents in different oncologic patients. However, it is currently unclear how DNA-PKcs inhibitors exacerbate the cellular sensitivity to chemotherapy and which oncologic patients may benefit from these drugs as adjuvant therapy.

Hypothesis

In this research proposal, we will focus on a non-canonical function for DNA-PKcs kinase in preventing genomic Single Strand Break (SSB) accumulation. Consequently, I have found that impaired DNA-PKcs function generates a novel cellular vulnerability, which is the sensitivity to PARP inhibitors. The DNA-PKi-induced vulnerability to PARPi is further exacerbated in cells lacking BRCA1/2 function and can be therapeutically exploited to overcome acquired PARPi resistance in breast and ovarian cancer patients.

Aims

Throughout this research proposal, we will map the genome-wide localization of SSB in cells lacking DNA-PKcs function and functionally characterize these genomic subdomains. Moreover, we will narrow down to individual DNA-PKcs phosphorylation targets involved in SSB suppression and PARPi resistance. Finally, we will establish high-grade serous ovarian carcinoma-derived organoids from patients with somatic and germline BRCA1/2 mutations characterized by resistance to the clinically-approved PARPi Olaparib. Using these models, we will translate the accumulated knowledge and rationally use DNA-PKcs inhibitors to restore PARPi sensitivity.

Experimental design

To achieve my research aims, we will initially combine the ChIP-Seq and S1 END-Seq techniques to profile the genomic subdomains occupied by DNA-PKcs and map the genomic sites where DNA-PKcs suppresses SSB accumulation. To identify the precise DNA-PKcs effectors involved in SSB suppression and PARPi resistance, we will use phosphoproteomics to discover new DNA-PKcs phosphorylation targets in PARPi-resistant mammary tumor cells and then we will functionally and genetically characterize how DNA-PKcs-mediated phosphorylation regulates protein's function. Finally, we will establish de novo 3D patient-derived organoid models from BRCA-mutated high-grade serous ovarian carcinoma patients with acquired PARPi resistance and use DNA-PKcs inhibitors to overcome PARPi resistance.

Expected results

Results from this research action are expected to reveal unprecedented roles for DNA-PKcs in genomic

stability different from its canonical role in DNA repair. With our designed proteo-genomic approach, I expect to characterize a new signaling mode for DNA-PKcs and identify previously unknown phosphorylation targets important for SSB suppression and PARPi resistance. Finally, based on our preliminary results obtained with PARPi-resistant mammary tumor cells, I expect likewise we will be able to overcome PARPi resistance in patient-derived ovarian cancer organoids lacking BRCA1/2 function with the use of DNA-PKcs inhibitors.

Impact on cancer

PARPi resistance is an emerging clinical challenge, particularly for germline mutated BRCA1/2 patients with recurrent breast and ovarian cancer. In this proposal, we have used DNA-PKcs inhibitors as a novel approach to overcome PARPi resistance in BRCA1/2-deficient tumors. I believe this new concept represents a breakthrough contributing to improve the quality of life of several oncologic patients in the coming years.

Giuseppe Ercolano **MFAG - 2021**

Università degli Studi di Napoli "Federico II"

The role of gasotransmitters in regulating ILC functions in cancer

Background

Cancer is a leading cause of death worldwide, accounting for more than 8 million deaths per year. Cancer immunotherapy represents today the most promising anti-cancer strategy since the development of the first chemotherapies. However, primary or secondary resistance to therapy represents the major drawback in a large number of patients. Recent findings suggest that understanding the tumor microenvironment (TME) will be key to optimize immunotherapies. In that context, cytokine secreting cells are central players in modulating positive and negative signals in the TME. A recently described family of rare, but potent cytokine secreting cells, called Innate Lymphoid Cells (ILCs), is emerging as key regulator of immunity. ILCs mediate tumor immune responses by displaying both pro and anti-tumor effects according to the tumor type. Nevertheless, little is known on the key mediators that influence their pro- or anti-tumorigenic behavior. Among the different component of the TME, gasotransmitters (nitric oxide, carbon monoxide and hydrogen sulfide) and their synthesizing enzymes, are emerging as key modulator of tumor immune responses and these pathways where shown to affect cancer progression.

Hypothesis

ILCs are primarily tissue-resident cells, that respond swiftly and potently to innate stimuli. In this context, ILC function is regulated by the different components of the TME. Likewise, gasotransmitters are emerging as key TME components affecting cancer progression. Thus, our hypothesis is that by dissecting and manipulating gasotransmitter pathways in ILCs we could switch TME-immunosuppression and promote anti-tumor immunity, favoring cancer elimination.

Aims

The aim of this project is to decipher the role of gasotransmitters in ILC biology in tumor immunity, to identify potential new therapeutic targets for cancer patients. In detail, we will:

1) Dissect at the single cell level the gasotransmitter pathways in ILCs in healthy donors and cancer patients.
2) Functionally characterize the gasotransmitter pathways in vitro and in vivo in ILCs, gaining valuable insight into the regulation of ILC by these gas mediators in cancer.
3) Target gasotransmitter pathways in ILCs in relevant tumor mouse models to unravel potential therapeutic applications in patients

Experimental design

Both human and murine studies will be employed to define the role of these new metabolic pathways in modulating ILCs activity/plasticity in cancer settings.

Expected results

This project could unveil the first evidence that gasotransmitter pathways have a critical role in ILCs,

contributing to tumor immunity. We expect gasotransmitter enzymes to influence ILC-mediated tumor immunity by modulating their effector functions and/or plasticity. Moreover, by the direct validation of our observations in relevant in vivo tumor models we expect to define the ILC-gasotransmitter axis as an attractive and innovative therapeutic target for cancer.

Impact on cancer

Search for therapeutic options to target innate immunity, e.g. ILCs and natural killer cells, in cancer patients is a highly active research field. Our project perfectly fits in this niche and is expected to provide insight on a novel druggable pathway, the one of gas mediators, in ILCs in patients. This strategy might prove synergistic with other therapeutic options and help to overcome resistance to immunotherapies.

Michela Faleschini **MFAG - 2024**

I.R.C.C.S. Materno Infantile Burlo Garofolo

Antisense oligonucleotides (ASOs)-based therapy: hope for Fanconi anemia patients affected by splicing mutations

Background

Fanconi anemia (FA) is a rare genetic DNA repair deficiency syndrome characterized by chromosomal instability resulting in late onset bone marrow failure and high cancer risk. FA arises from loss of function mutations in at least 22 genes, with FANCA mutations accounting for 80% of cases. Among them, 20% are splicing mutations that disrupt gene function. Currently, the only option for FA patients is hematopoietic stem cell transplantation (HSCT) which only helps to partially restore the hematological phenotype with no effect on reduction of the cancer risk.

Hypothesis

Over the past ten years, new therapies based on the ability of antisense oligonucleotides (ASOs) to modulate splicing have been approved by the Federal Drug Administration (FDA) or the European Medicines Agency (EMA) to treat previously untreatable genetic diseases.

This led us to hypothesize that ASOs could represent a promising approach to correct aberrant splicing in FA patients affected by cryptic splicing mutations.

Aims

The main aim of this project is to explore if ASO-based therapy could become a novel approach for treating Fanconi anemia (FA) patients with cryptic splicing mutations.

Experimental design

We will achieve the aim of the project as follows:

WP1: Assessing the efficacy of ASO(s) in rescuing the phenotype on lymphoblastic cell lines derived from a FA patients affected by FANCA c.2778+83C >G (pilot mutation)

WP2: Testing lipid nanoparticles (LNPs) as ASO(s) delivery strategy through activity and toxicity assays in vitro and in vivo

WP3: Generating a EGFP splice-switching reporter mouse model

WP4: Evaluating the ASO(s)-LNPs bioactivity in different tissues and organs in the EGFP splice-switching reporter mouse model generated in WP3

WP5: Expanding the ASO-based approach optimized for the pilot mutation to other cryptic splicing mutations affecting FA genes.

Expected results

We expect to set up an optimized workflow for the development of mutation-specific targeting ASOs and assess the toxicity and bioactivity of ASO(s)-LNPs in vitro and in vivo.

Impact on cancer

The possibility of applying ASO-based approach to FA patients affected by cryptic splicing mutations may

offer the first real treatment aimed to stop disease progression, delaying or even preventing not only bone marrow failure but also, for the first time, tumor development.

Luca Fava **Bridge Grant - 2024**

Università degli Studi di Trento

Unravelling the intricacies between whole genome doubling, p53, and the m6A writer complex

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Background

The p53 tumor suppressor, recognized for decades as a key regulator in curbing cell proliferation with ploidy abnormalities, is particularly important to shape the fate of tetraploidy or Whole Genome Doubling (WGD), a condition present in 30-37% of human cancers. Unsurprisingly, TP53 mutations are more prevalent in this large tumor subset. Despite the absence of robust experimental support for a tetraploidy checkpoint, our research has contributed to establishing that mammalian cells utilize mature centrosome count as an indirect measure of their ploidy status. Centrosome amplification triggers PIDDosome-dependent cleavage of MDM2, the primary inhibitor of p53, pivotal for p53 activation.

Concurrently, m6A, or N6-methyladenosine, emerges as a prevalent RNA modification regulating mRNA stability, splicing, and translation. Governed by the m6A writer complex, a protein assembly centered around its catalytic subunit METTL3, this modification is a target for pharmacologic inhibition with significant therapeutic implications. While suppressing METTL3 bears potential for diminishing cellular fitness, it also enhances interferon signaling and anti-tumor immunity. Intriguingly, recent findings indicate a physical association between the m6A writer complex and the p53 protein, which results in a reinforced p53 functional output. This dual role of the m6A writer, acting as a guardian against unscheduled immune responses and a supporter of p53, presents a therapeutic challenge, necessitating a profound exploration of this intricate interplay.

Hypothesis

Our hypothesis posits an unexplored interplay between p53 and the m6A writer during WGD, suggesting a joint influence on cellular fate. We propose that WGD alters the m6A writer's chromatin association, leading to transcriptome-wide changes in m6A modification and gene expression.

Aims

This project aims to i) comprehensively examine the global interplay between p53 and the m6A writer during WGD in two distinct conditions: a) the healthy condition (WT p53) and b) the malignant condition (mutant p53). Additionally, ii) we seek to identify resistance mechanisms to METTL3 inhibitors using PIDDosome-specific tools, thereby enhancing our understanding of the complex interactions within these crucial cellular pathways.

Experimental design

For Aim i), we will integrate m6A writer ChIP-Seq and transcriptome m6A profiling in both WT p53 and mutant p53 conditions. Aim ii) will involve base editor screens to uncover mutations in the m6A writer associated with resistance to METTL3 inhibitors.

Expected results

Anticipated outcomes include i) a comprehensive understanding of the p53-m6A writer interplay during WGD, uncovering m6A's role in gene expression regulation, and ii) a new CRISPR resource focused on an emerging drug target, guiding future investigations.

Impact on cancer

This work i) informs patient stratification for METTL3 inhibitory regimens based on WGD and TP53 status, and ii) provides insights into mutations promoting METTL3 inhibitor resistance, shaping future therapeutic strategies and expediting clinical translation of METTL3 inhibitors.

Roberto Ferrara **Next Gen Clinician Scientist - 2024**

Università Vita-Salute San Raffaele

Hyperprogression upon PD-(L)1 inhibitors alone or with chemotherapy in PD-L1≥50% NSCLC: a biomarker guided phase 2 trial

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Background

An acceleration of tumor growth, known as hyperprogressive disease (HPD) has been described in 14-26% of patients with non-small cell lung cancer (NSCLC) treated with single agent (SA-ICI) ICI in 2nd or further lines. Of note, the addition of platinum-based chemotherapy (PCT) to ICI in 1st line seems to prevent HPD and early mortality. We have recently reported that ≥ 30.5% of circulating CD10- low density neutrophils (LDNs) could pre-identify among NSCLC patients with PD-L1 tumor expression ≥ 50%, those who are at high risk of HPD upon 1st line SA-ICI. In patients being at high risk of HPD due to CD10- LDNs ≥ 30.5%, treatment with PCT+ICI not only prevented HPD but also significantly reduced circulating immature neutrophils below the threshold.

Hypothesis

In metastatic NSCLC patients with PD-L1 TPS ≥50%, a level of circulating immature (CD10-) LDNs ≥ 30.5% confers a high risk of HPD upon 1st line single-agent ICI. The combination of PCT+ICI in this setting, by modulating immune suppressive neutrophils subsets, could prevent the occurrence of HPD and ultimately improve survival outcomes.

Aims

The main aims of the present proposal are: 1) to demonstrate that 1st line PCT+ICI prevents HPD in patients with NSCLC and PD-L1 TPS ≥50% defined at high risk of HPD according to the level of circulating CD10- LDNs, 2) to determine how HPD features are favorably modulated by the addition of PCT to ICI.

Experimental design

The experimental plan is divided in 2 work packages (WPs): WP1 will focus on patients screening by flow cytometry and on enrollment of 78 patients at high risk of HPD in the randomized phase. WP2 by using high through put technologies (i.e.; single cell RNA sequencing, spatial transcriptomic, digital pathology, liquid biopsy) on both blood and tissue samples collected at baseline and, whenever possible, longitudinally during treatments, will assess how HPD features are differently modulated by treatment type (ICI or PCT+ICI).

Expected results

In patients at high risk of HPD due to ≥30.5% circulating CD10- LDNs, PCT+ICI significantly reduce the rate of HPD compared to SA-ICI and favorably modulate CD10- LDNs in the blood. HPD circulating and tissue features are differently modulated by ICI or PCT+ICI.

Impact on cancer

HYPERBOLIC is the first randomized study comparing 1st line SA-ICI versus PCT-ICI, which adopts HPD prevention as primary endpoint and addresses this question in a biomarker-based design fashion. By measuring the speed of growth during ICI, HYPERBOLIC will pave the way to novel clinical trials where tumor

kinetics before ICI start should be taken into account to better classify patterns of response or progression. HYPERBOLIC by potentially enriching HPD in biomarker selected patients could help to discover biological mechanisms of HPD in a larger and homogenous population and to assess the role of chemotherapy in modulating HPD features. In Italy, only in 2022 ~43.900 new cases of NSCLC have been diagnosed; considering that HPD can occur in up to ~15% of them, a PCT-ICI escalating treatment strategy in HPD high-risk patients, could potentially save hundreds of lives.

Valentina Fragliasso **MFAG - 2023**

Azienda Unità Sanitaria Locale di Reggio Emilia IRCCS

Preserving DNA: investigating the genomic role of HELLS in the development and progression of T-cell Lymphomas

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Background

T-cell Lymphomas (TCLs) are a rare and heterogeneous group of non-Hodgkin lymphomas with aggressive courses and poor prognoses. Mechanisms underlying their progression are not fully elucidated limiting the development of targeted therapeutic strategies. A high transcriptional rate is required to support massive cancer cell proliferation but the transcription is a dangerous process that facilitates DNA damage and genomic instability. We recently identified HELLS -a ubiquitously expressed DNA-helicase deputed to DNA structure resolution- as a vulnerability of TLCs demonstrating that HELLS orchestrates a transcriptional program, impacting on survival of TCLs.

Hypothesis

We hypothesize that HELLS plays a bi-modal transcriptional function in TCLs. By binding a subset of immune-specific promoters, HELLS primes the accessibility of the genome to TFs coordinating immune-related transcriptional programs favoring RNAPII recruitment and transcriptional activation. Instead, by avoiding DNA-topological conflicts, this helicase facilitates RNAPII progression contributing to support high-rate transcription while supervising genomic stability.

Aims

The goals of this proposal are

1) shed light on the molecular mechanisms through which HELLS integrates transcription and DNA maintenance on cancer-specific sites supporting TCLs progression (AIM 1-2).

2) Identify and develop specific inhibitors to exploit the synthetic lethality between HELLS inhibition and drugs approved for the treatment of TCLs (AIM3).

Experimental design

Using the combination of "omics" (RNA-sequencing, ChIP-sequencing, DRIP-sequencing and DSP) and molecular functional approaches we will define: i) its cooperation network in controlling immune response, ii) the role of HELLS in genome integrity and iii) the mechanisms through which HELLS balances the transcription and DNA-repair. Performing CRISPR and synthetic lethality screenings, we will identify potential drugs with synergistic activity to HELLS depletion. Furthermore, we will identify and characterize selective anti-HELLS compounds by in silico approach followed by in vitro and in vivo functional experiments.

Expected results

Obtained results will help to elucidate the mechanisms through which HELLS sustains TCL progression. Furthermore, by identifying small molecules with anti-HELLS properties and evaluating their potential in reducing cell proliferation, we will provide proof of principle data to support the use of anti-HELLS compounds as anti-lymphoma agents.

Impact on cancer

Although important steps have been made in defining the pathobiological mechanisms of TCLs, many shadow areas and unresolved questions remain, leaving a significant portion of patients without the most adequate therapies. At the crossroads between replication, transcription, and DNA repair, HELLS is an attractive target for developing novel anti-cancer treatments for hematological malignancies. Dissecting the role of HELLS in promoting TCL progression, we will gain novel in-depth insights into the pathogenesis of TCLs. Integrating "omics" data with functional experiments, we will approach these diseases from a different and translational perspective generating new milestones in the biology and the treatment of TCLs, and candidating HELLS as a novel therapeutic target. Moreover, being HELLS deregulated in many tumors, the knowledge generated by this study will be relevant to other cancer models.

Giorgio Gandaglia **MFAG - 2024**

Università Vita-Salute San Raffaele

Biological Pathways and Next-Generation Imaging Features Predicting Prostate Cancer Progression in Active Surveillance

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Background

Active surveillance (AS) is the treatment of choice for low-risk prostate cancer (PCa) to avoid unnecessary treatments. Despite stringent criteria, about 30% of AS patients experience progression within a year, and 70% receive definitive treatments within 10 years. Accurate biomarkers for identifying AS candidates who need immediate radical treatment are lacking. Understanding the biological landscape of low-risk PCa assessing genomic alterations, gene expression patterns, cell population spatial arrangements, and microenvironmental features associated with progression could lead to novel biomarkers. These, combined with next-generation imaging (i.e., PSMA PET) could develop accurate prediction tools for identifying AS patients at high risk of progression who need upfront treatment.

Hypothesis

The combination of novel biomarkers identified through an in-depth assessment of biological pathways associated with progression and next-generation imaging features could help characterizing the landscape of PCa and tailor individualized treatment strategies.

Aims

1. Develop and validate a model combining clinical features with somatic mutations, PSMA-PET quantitative parameters, and PSMA expression at IHC to identify patients who will experience upgrading at 1-year follow-up.
2. Assess the impact of genetic heterogeneity on the risk of upgrading
3. Explore the role of gene expression patterns, spatial arrangement of cell populations, and microenvironmental features in PCa progression.
4. Investigate the role of PSMA PET imaging in identifying AS patients who will progress.

Experimental design

Patients with low-risk PCa (biopsy ISUP Grade Group [GG] 1, PSA ≤10 ng/ml, and cT1-cT2a) considered for AS will represent the study cohort. All patients will be assessed with Whole Exome Sequencing (WES) and Spatial Transcriptomics on diagnostic biopsy; PSMA-PET/MRI; Multiplex IHC to quantify the predominant PSMA expression and biomarkers according to spatial transcriptomics findings.

Primary outcome: upgrading defined as biopsy ISUP GG≥2 at 1-year confirmatory biopsy.

Results of WES, PSMA PET/MRI, spatial transcriptomics, and multiplex IHC will be compared between patients experiencing upgrading vs. those who are not. The biological signatures will be combined with clinical variables and PSMA-PET quantitative parameters to create a model to predict upgrading. We aim to enhance the discrimination of available models from 70% to 85%. Given a prevalence of 1-year upgrading of 30% in our cohort, we will enroll 74 participants, ensuring a statistical power of 80% and a significance level of 0.05.

Expected results

The in-depth characterization of biological and next-generation imaging features associated with upgrading will lead to the development of a tool to select AS candidates. This tool will enhance oncologic control by treating men at higher risk of progression upfront, enable personalized decisions, and reduce healthcare costs.

Impact on cancer

The extensive characterization of low-risk PCa using molecular biology and next-generation imaging features allows for the identification of men who should receive curative-intent therapies upfront and de-intensify follow-up procedures for those with indolent disease. This would streamline the current AS diagnostic pathway and reduce associated costs and side effects. The availability of reliable tools to identify patients at higher risk of progression would increase the adoption of AS, thereby reducing the risk of overtreatment. Ultimately, this would reduce the burden of low-risk PCa and result in substantial savings for healthcare systems.

Alice Giustacchini **Start-Up - 2024**

Fondazione Human Technopole

Tailoring Precision Immunotherapy to Paediatric Acute Myeloid Leukemia

Background

Paediatric Acute Myeloid Leukaemia (AML) presents significant treatment challenges due to its heterogeneity and poor response to conventional therapies. Recent advancements in CAR T cell therapy have shown promise in other haematologic malignancies but are yet to be fully realised for AML due to the complexity of targeting leukemic stem cells (LSCs) without harming healthy haematopoietic stem cells.

Hypothesis

We hypothesise that a multi-targeted CAR T cell approach, informed by in depth transcriptomic and immunologic profiling of paediatric AML, may effectively target the LSCs while sparing normal cells, leading to improved therapeutic outcomes. Additionally, we propose that insights gained from existing multi-targeting strategies in other diseases can be leveraged to refine and optimise CAR T-cell designs for AML, enhancing efficacy and reducing the potential for relapse.

Aims

We will establish molecular profiles of AML relapse by single-cell transcriptomic analysis, aiming to uncover resistant cell subsets and provide actionable insights into therapeutic escape mechanisms (AIM1). We will next complement these findings by elucidating the crosstalk between AML LSCs and the immune system through 3D organoid modelling, aiming to identify the signalling pathways that underlie immune evasion and leukaemia persistence (AIM2). We will next g computational tools to map antigen combinations uniquely expressed by AML cells, thus enabling precision targeting while preserving healthy cells (AIM3).
Finally, we aim to identify persistence markers in multi-targeting CAR T trial for B-ALL (CD22/CD19, UCL), to refine therapeutic strategies to enhance treatment efficacy when designing multi-targeting approaches for AML CAR T cell treatment (AIM4).

Experimental design

We will integrate single-cell RNA sequencing with long-read sequencing to capture leukaemia-specific transcript isoforms, critical for identifying unique CAR T cell targets. Concurrently, CITE-seq will be employed to quantify surface protein levels, linking RNA data to actual protein expression on leukaemia cells, which is vital for precise target validation.
Our experimental approach also includes a 3D bone marrow organoid model that mimics the human marrow environment, enabling detailed analysis of AML-immune cell interactions and potential immune dysfunction pathways. Additionally, by combining ATAC-seq with mRNA profiling of dual CAR T cell treated B-ALL patients, we will explore the epigenetic landscape alongside gene expression changes to elucidate relapse mechanisms and refine multitargeting CAR T-cell strategies for AML.

Expected results

Through the understanding of AML phenotypic, immunologic, and genomic complexity, we will inform the

design of CAR T cell approaches tailored to paediatric AML. I aim to develop a method that can be widely applied to the analysis of leukaemia progression and translated to future personalised treatment choices.

Impact on cancer

The potential impact is twofold: on one side we will advance understanding of AML complexity and immune system interactions, pinpointing cells resistant to therapy and informing strategies to counteract chemotherapy escape (AIM1-3). On the other hand, it will allow us to model the impact of multi-targeted CARs, setting the stage for designing optimal multitargeting strategies for AML therapy (AIM4). While focused on paediatric AML, this framework is designed to be broadly applicable to other haematological malignancies, thereby broadening the impact of this research in the field of oncology.

Giovanna Grimaldi **SIS - 2023**

Consiglio Nazionale delle Ricerche

Overcoming breast cancer resistance to hormone-based therapy by targeting the PARP12 enzyme

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Background

Breast cancer is one of the leading types of cancer in women; in approximately 70% of total cases, proliferation and growth of breast cancer cells rely on estrogen receptor (ER) signaling, therefore hormone-based therapy is the standard of care for these tumors. Despite its high successful rate, still a substantial fraction of patients dies due to the acquisition of drug resistance.

ADP-ribosylation is a post-translation modification catalyzed by PARP enzymes that regulates essential cellular processes, often altered in diseases. PARP12 - a member of the family- has been associated to the onset of drug resistance in ER+ breast cancers, making this enzyme a promising drug target. The molecular basis underlying its involvement in the acquisition of resistance are unknow to date.

Hypothesis

The main hypothesis of the present proposal is that PARP12 contributes to promote survival of breast cancer cells, possibly by intervening in the PI3K/Akt/mTOR pathway, and hence counteracting PARP12 enzymatic activity may be beneficial in the treatment of ER+ breast cancers. Multiple biological mechanisms concur to the development of resistance, including mutation in the ESR1 gene, alterations of the PI3-Kinase/Akt/mTOR pathway or of the cyclin-dependent kinases 4/6 pathways, for which combinatorial therapies using targeted drugs have proven to ameliorate survival. Here, we hypothesize that the PARP12-mediated signaling is part of those pathways activated in non-responding patients, therefore targeting PARP12 is here presented as a novel breast cancer therapeutical strategy.

Aims

Our main aims are i) to dissect the molecular basis of the PARP12-mediated ADP-ribosylation pathways underlying cell survival of breast cancer cells; ii) to investigate the interplay between PARP12- and estrogen receptor-mediated signaling; iii) to identify drug-like PARP12 selective inhibitors for in vivo use as anticancer agents in breast cancer tumors.

Experimental design

The project is organized in 3 main tasks. First is the dissection of the PARP12-mediated signaling in breast cancer cell models by: a) analyzing the effects of PARP12-mediated ADP-ribosylation on Akt activation and functions and b) identifying the ADP-ribosyl proteome of PARP12 "sensitive" models. Second is the study of an interplay between PARP12 and ER□-dependent transcription, by analyzing the breast cancer transcriptome and the regulation of the receptor multiprotein complex activity. Third is the development of PARP12 specific inhibitors that impairs breast tumor growth.

Expected results

We expect to delineate the molecular basis of the PARP12-dependent cell survival in a subset of breast

cancer tumors, and to develop PARP12 inhibitors to be proposed as novel drug, eventually for combinatorial therapies.

Impact on cancer

To date, approximately 40% of endocrine-resistant breast tumors hold known genomic alterations, while the remaining 60% still rely on undiscovered alterations. According to statistics, breast cancer still represents the first cause of death in women. High levels of PARP12 in ER+ breast cancer correlate with a poor survival. With the execution of this project, we expect to provide the basis for the development of a novel treatment for breast cancer tumors relying on altered PARP12-driven pathways.

Roberto Iacovelli **Next Gen Clinician Scientist - 2024**

Fondazione Policlinico Universitario Agostino Gemelli IRCCS

Clinical and humoral impact of primary tumor ablation in metastatic renal cell carcinoma treated with immunotherapy.

Background

Cytoreductive nephrectomy (CN) for metastatic renal cell carcinoma (mRCC) has been always a topic of interest because it removes the primary tumor with bleeding and pain control and might minimize the risk of future metastatic seeding from primary tumour. This also removes the potential source of immunosuppressive or tumor-promoting growth factors. No prospective data are available about the role of CN in patients eligible for or while on treatment with the immune checkpoint inhibitor-based therapies (CPI; anti-PD1 or anti CTLA4), for advanced disease.

Hypothesis

When directed on primary tumor, either surgery or RT can remove a possible source of immunosuppressive or tumor-promoting growth factors and finally decrease the risk of metastasis. Based on this assumption, the ablation of primary tumor during treatment with anti-PD1-based therapies can improve the overall survival (OS) and change the proteome favouring a positive milieu for CPI-induced immune response.

Aims

This study aims to demonstrate that patients who receive deferred CN while on treatment with anti-PD1 based therapies can survive more compared to those who did not. It can also describe the role of RT for patients with primary tumor eligible to ablative treatment as well as the changes in the proteome after CN or RT.

Experimental design

A randomized multicentre phase II study will be designed to show the superiority of deferred CN when added to anti-PD1-based therapies compared to anti-PD1-based therapies alone. Patients affected by clear cell mRCC with primary tumor and intermediate or poor IMDC prognosis undergoing anti-PD1-based therapies from at least 24 and no more than 36 weeks will be enrolled. Patients with the maximum tumor diameter (MTD) of the primary tumor greater than 7 cm will be randomized 1:1 to continue the medical therapy with or without surgery, those with MTD up to 7 cm will be randomized 1:1:1 to continue the medical therapy with surgery or radiotherapy or nothing. All patients will continue the ongoing medical treatment until progression of disease or unacceptable toxicity. Surgery on primary tumor can be radical or partial nephrectomy as clinically indicated. The OS will be evaluated from the beginning of the medical treatment. A plasma sample will be stored at the time of randomization for all patients and after height weeks from surgery or radiotherapy to assess the proteome.

Expected results

This study has power to show if CN is able to improve the OS of mRCC patients and can offer some evidence for the use of RT (with CPI) in the same population. Description of changes in the proteome profile may

explain the biological effects of surgery or radiotherapy on primary tumor.

Impact on cancer

The OS is recognized as the best endpoint for clinical trials design for cancer patients and, if achieved, this study can significantly improve the current landscape of mRCC. This is of relevance considering that CN is a standard surgical procedure with low risk of postoperative complications and without significant delay of the medical treatment.

Gianluca Ianiro **Next Gen Clinician Scientist - 2024**

Università Cattolica del Sacro Cuore

A gut microbiome-based diagnostic tool for the screening of colorectal cancer

Background

Colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer death worldwide. Population-based screening programs for populations at average risk are effective in reducing the incidence and mortality of CRC. The fecal immunochemical testing (FIT), the reference screening test in the majority of countries, is still encumbered by diagnostic issues, with both missed adenomas and, mainly, unnecessary colonoscopies. Therefore, novel non-invasive biomarkers are advocated. The imbalance of gut microbiome appears to be involved in the pathogenesis of CRC. Moreover, recent studies have discovered common microbial signatures able to reproducibly discriminate between patients with CRC and healthy controls. International guidelines have recently advocated the use of microbiome-based biomarkers for the screening of CRC, but the evidence for their application in clinical practice is not yet available.

Hypothesis

Based on the association of microbiome signatures and CRC, we hypothesize that 1) the gut microbiome of FIT-positive patients undergoing CRC screening is able to predict CRC and advanced colorectal adenomas (ACAs); 2) based on these findings, a microbiome-based diagnostic tool will improve the diagnostic performances of FIT for the detection of CRC and ACAs.

Aims

Our aim is to build a gut microbiome-based diagnostic tool to improve the detection of CRC and ACAs. Specifically, we aim to:

1) Collect clinical data and stool samples in a large cohort of FIT-positive patients undergoing screening colonoscopy
2) Comprehensively characterize the gut microbiome of enrolled subjects.
3) Build a microbiome-based diagnostic tool able to identify CRC and ACAs.

Experimental design

A large (n=1202) cohort of FIT-positive subjects undergoing screening colonoscopy will be enrolled, based on sample size calculation and including a validation group. Stool samples and clinical data will be collected for each patient. After colonoscopy endoscopic and histopathological data will be collected. Gut microbiome will be analyzed by shotgun metagenomics. The performance of the FIT in predicting the endoscopic outcomes (CRC or ACAs) will be evaluated by the positive predictive value. Clinical, endoscopic and microbial data will be combined through statistical and machine learning algorithms to identify specific microbial biomarkers associated with CRC and develop a new diagnostic tool, based on a scoring system. This tool will be validated. and its diagnostic performances will be compared with traditional screening methods.

Expected results

We expect to retrieve microbiome data from a large cohort of FIT-positive subjects undergoing screening colonoscopy, and to associate significantly and reproducibly the presence of CRC and ACAs with specific microbial signatures. These features will be combined to build an innovative microbiome-based diagnostic tool, based on a scoring system, that will provide early detection of CRC and ACAs.

Impact on cancer

Our results would revolutionize CRC screening, reducing unnecessary colonoscopies and increasing the likelihood of early CRC diagnosis. More widely, our findings would provide a mindset shift in the clinical and scientific community, as they would pave the way to a new scenario of microbiome-based diagnostics for cancer.

Andrea Idili

Università degli Studi di Roma "Tor Vergata"

Translating monoclonal antibody pharmacokinetics into clinical practice: toward personalized immunotherapy

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Background

Monoclonal antibodies (mAbs) have revolutionized cancer therapy, but their efficacy is limited by significant inter-individual variability in patient response. This variability, influenced by tumor heterogeneity, immune response, and patient characteristics, leads to unpredictable pharmacokinetics and forces clinicians to administer high doses of mAbs, increasing treatment costs. Monitoring mAb concentrations in patients could enable personalized dosing, but current bioanalytical methods are complex, costly, and unsuitable for point-of-care use, hindering their implementation in clinical practice. As a result, the variability of mAbs remains poorly understood, hampering personalized immunotherapy.

Hypothesis

Imagine a future where monitoring life-saving immunotherapy drugs is as easy as testing your blood sugar at home. We hypothesize that a point-of-care device capable of rapidly and accurately measuring mAb levels could revolutionize cancer treatment by providing patients and physicians with real-time, personalized data. This innovative tool would not only simplify monitoring, but also enable tailored dosing adjustments, optimize therapeutic decisions and ultimately improve patient outcomes. In addition, by simultaneously tracking anti-drug antibodies (ADA) and total IgG, this device could provide critical insight into the factors that influence treatment response, paving the way for a new era of precision medicine in oncology.

Aims

Leveraging my expertise in DNA nanotechnology, in vivo biosensing, and point-of-care (PoC) platforms, I propose a groundbreaking approach to develop a biomedical device for monitoring clinically relevant antibodies (mAbs, ADA, IgG). The overarching goal of my research program is to create a rapid (< 15 min), single-step, affordable, and versatile biosensing technology capable of quantifying multiple antibodies in a finger-prick blood sample directly at the point-of-care. Specifically, I will focus on the quantification of therapeutic mAbs (trastuzumab, bevacizumab, rituximab), their associated anti-drug antibodies (ADA), and human IgG. This will be achieved by harnessing the adaptability and programmability of DNA in conjunction with the advantages of electrochemical platforms to develop innovative electrochemical DNA-based (eDNA) sensors.

Experimental design

This research aims to revolutionize immunotherapy by developing a portable, low-cost device capable of rapidly detecting mAbs, ADA, and total IgG directly in blood. This will be achieved through the creation of programmable eDNA sensors, utilizing aptamers and DNA scaffolds as recognition elements for specific antibody detection. These sensors will be engineered with allosteric mechanisms to finely tune their sensitivity, enabling precise monitoring of antibody concentrations across a broad therapeutic range. The integrated platform will combine these sensors with a miniaturized electrochemical device and open-source

software, paving the way for personalized immunotherapy and enhanced patient care.

Expected results

This research aims to revolutionize immunotherapy by developing a PoC eDNA sensor platform enabling personalized treatment through rapid, multiplexed antibody detection and quantification, direct measurement of pharmacologically active drug fractions, and real-time monitoring.

Impact on cancer

This research will yield an innovative biomedical device enabling personalized immunotherapy. The device will allow for real-time monitoring of therapeutic antibodies, anti-drug antibodies, and total IgG, offering unprecedented insights into patient responses and treatment efficacy. This will empower clinicians to tailor dosing regimens, predict adverse reactions, and improve treatment selection, ultimately enhancing the cost-effectiveness of immunotherapy and making it more accessible to patients.

Paola Infante **MFAG - 2021**

Università degli Studi di Roma "La Sapienza"

Defining the role of the cytoskeleton regulator inverted formin INF2 in medulloblastoma tumorigenesis

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Background

Medulloblastoma (MB) is the most common and aggressive pediatric brain malignancy. The high heterogeneity of MB makes extremely difficult determining a successful therapy. Among MB's molecular subgroups, Sonic Hedgehog (SHH) is the most abundant and genetically understood. SHH-MB is characterized by genetic alterations of key components of HH signaling, a developmental pathway emerged as an attractive therapeutic target for MB treatment. However, the molecular mechanisms driver of SHH-MB still require to further be unveiled to design more effective therapy. Recently, we identified INF2, a formin involved in the regulation of actin and microtubule cytoskeletal dynamics, as a putative negative regulator of HH signaling that deserves further investigation regarding its involvement in MB tumorigenesis.

Hypothesis

We believe that the regulation of the HH pathway mediated by INF2 represents a novel and relevant aspect for MB biology, which could further illuminate on the role of cytoskeleton in HH-driven tumorigenesis. Indeed, our preliminary findings unveil a negative role of INF2 in controlling HH signaling and a drastic decrease of INF2 protein levels in SHH-MB. Moreover, INF2 protein stability is increased by FBXW7-mediated ubiquitylation, a tumor-suppressor highly mutated in MB. We hypothesize that the loss of INF2 expression, due to mutations of FBXW7, might play a key role in SHH-MB tumorigenesis.

Aims

The project is organized in three aims:

- decipher INF2 role in the regulation of HH signaling
- characterize the mechanisms that control INF2 function in HH signaling
- study the biological role of INF2 in HH-dependent cell growth and MB tumorigenesis

Experimental design

TASK 1. Characterization of the role of INF2 in the regulation of HH signaling.

- Molecular characterization of INF2 in controlling HH pathway
- Evaluation of INF2-dependent activity on microtubule and mitochondria dynamics in HH signaling

TASK 2. Characterize the mechanisms that control INF2 function in the HH pathway.

- Study of FBXW7-mediated ubiquitylation of INF2
- Identification of INF2 interactors involved in its ubiquitylation
- Study of FBXW7 mutants in the regulation of INF2 ubiquitylation

TASK 3. Study of the biological role of INF2 in HH-dependent cell growth and MB tumorigenesis.

- Biological in vitro and in vivo evaluation of INF2 effects on HH-dependent cell and MB growth

- Characterization of INF2 in MB onset and tumorigenesis

Expected results

We expect our efforts will provide: i) a novel understanding of regulatory mechanisms mediated by INF2 in physiological and pathological HH-dependent signaling; ii) an explanation as to why mutations in FBXW7 are found in SHH-MB; iii) a definition of the role of cytoskeleton in HH-driven tumorigenesis.

Impact on cancer

MB is a leading cause of cancer-related deaths in children and a definitive cure is elusive. A detailed understanding of MB biology is fundamental to advance more effective therapy. In this scenario, this project will pave the way to a novel area of investigation in HH signaling by involving cytoskeletal remodeling proteins in HH-driven MB biology. The characterization of INF2 as negative regulator of HH signaling and of its impact on HH-MB tumorigenesis could offer alternative targets for the future design of more successful therapeutic strategies for MB.

Alessandro Malara **MFAG - 2020**

Università degli Studi di Pavia

FIBROmeltIN: Targeting Fibronectin in Myelofibrosis

Background

Primary myelofibrosis (PMF) is a clonal myeloproliferative neoplasm characterized by excessive deposition of extracellular matrix (ECM) in bone marrow (BM), megakaryocyte hyperplasia, and splenomegaly. The identification of somatic "driver" mutations in JAK2, CALR, and MPL genes has contributed to a better definition of disease pathogenesis. Fibronectin (FN) is a major structural protein of BM ECM and exists in several isoforms through alternative splicing events. FN homeostasis is significantly unregulated in PMF as: I) FN expression and secretion are abnormal in mesenchymal stem cells (MSCs) of PMF patients and correlate with fibrosis; II) an alternatively spliced EDA FN isoform sustains megakaryocytosis, myelofibrosis and splenomegaly; III) increased expression of FN receptors and augmented binding avidity to FN promote JAK2V617F-megakaryocyte proliferation. These evidences attribute to FN a non-redundant role in the aberrant cross-talk between hematopoietic and stromal cells that inevitably assure the dysfunctional BM microenvironment during exacerbation of myelofibrosis.

Hypothesis

Our hypothesis is that FN is a key player in the interaction between malignant megakaryocytes and fibrosis-driving cells. Preliminary data in a mouse model of myelofibrosis suggest that the increased production and polymerization of BM FN is due to both local synthesis of alternatively spliced EDA FN by MSC/endothelial fibrosis-driving cells as well as incorporation and assembly of circulating plasma FN in the ECM. Once deposited, FN concurs to render the BM environment more permissive to megakaryocyte proliferation.

Aims

We propose an integrated gene/protein targeting strategy to prevent FN production and polymerization which are elevated in clinical and experimental myelofibrosis. To implement our strategy, we propose the following aims: in Aim 1 we will establish the in vitro efficacy of FN targeting to modulate the pro-fibrotic phenotype of MSCs and endothelial cells; in Aim 2 we will test whether interfering with stromal/endothelial FN expression/organization can refrain the abnormal JAK2V617F-megakaryocyte proliferation in co-culture systems; in Aim 3 we will determine the mechanism(s) of (I) inhibiting FN polymerization, (II) ablating FN expression and, (III) targeting the EDA FN splice variant expression in vivo.

Experimental design

Inhibition of FN polymerization will be achieved with a recombinant peptide that inhibits its cell-mediated assembly/polymerization. FN knockdown and forced EDA exon skipping in vitro will be performed with neutralizing targeted mRNA molecules (e.g. siRNA, modified U7snRNA). Antisense Oligonucleotides (ASOs) will be designed for FN gene expression silencing and to induce EDA exon skipping in vivo. Peptides and ASOs will be tested in two murine models of Thrombopoietin- and JAK2V617F-induced myelofibrosis, respectively.

Expected results

The primary outcomes of this project will be to assess whether preventing FN fibril assembly, ablation of its expression, or modulation of FN mRNA splicing, and combination thereof, will result in the reversal of the altered BM microenvironment and fibrosis in mouse models of myelofibrosis.

Impact on cancer

Median survival in PMF is estimated at approximately six years. The sole available targeted therapy for PMF, the JAK2 inhibitor Ruxolitinib, has contributed to relief from inflammatory symptoms and splenomegaly but rarely resolves BM fibrosis. Thus, successful completion of the proposed translational research will provide new strategies to prevent and diminish ECM dysregulation and fibrosis in PMF.

Nicola Manfrini **MFAG - 2021**

Fondazione "Istituto Nazionale Genetica Molecolare - INGM"

Dissecting the function and druggability of the intracellular networks regulated by the FAM46C oncosuppressor complex

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Background

Multiple myeloma (MM) is an incurable hematologic malignancy characterized by abnormal proliferation of plasmacells. Despite recent therapy advancements, the five-year survival rate is still below 50%, suggesting the need for better therapies and novel therapeutic targets. One of the most frequently mutated genes in MM is oncosuppressor FAM46C, whose expression is also drastically altered in several other tumors, including breast cancer. FAM46C localizes at the cytoplasmic side of the endoplasmic reticulum through interaction with FNDC3A, and here it re-routes lysosomes from the autophagic machinery towards exocytosis in turn causing, on one side, defective autophagic clearing of protein aggregates and apoptosis induction and, on the other, a drastic alteration of the secretome with effects on immune cell functionality.

Hypothesis

By working at the crossroads between intracellular trafficking and secretion, FAM46C has both cell-autonomous and non cell-autonomous effects. We hypothesize that unraveling this dualism of FAM46C functionality will lead to the discovery of druggable enzymes. Moreover, given evidence for involvement of FAM46C/FNDC3A in breast cancer, we hypothesize that the FAM46C complex works as an oncosuppressor also in breast malignancies.

Aims

We aim at identifying novel druggable targets for cancer therapy by defining, through genetic and biochemical approaches, the intracellular and extracellular networks regulated by the FAM46C oncosuppressor complex. In addition, we aim to study for the first time the oncosuppressor role of FAM46C in breast cancer.

Experimental design

Area 1 is focused on MM studies and is divided in two aims focused on defining: 1.1) how FAM46C-expressing MM cells affect the tumor microenvironment and 1.2) which intracellular networks are regulated by FAM46C. Aim 1.1 is divided in three smaller tasks in order to: analyze the secretome of MM cells expressing FAM46C (Task 1.1.1), define the protein-receptor repertoire of FAM46C expressing cells (task 1.1.2) and establish a 3D MM in vitro model to test FAM46C role in immunomodulation (Task 1.1.3). Aim 1.2 is divided in two tasks in order to: characterize the specific FAM46C-BCCIP interaction (task 1.2.1) and determine FAM46C genetic interactors (task 1.2.2). Both aims will define novel druggable targets. Area 2 revolves on breast cancer and is divided in two aims focused on: 2.1) verifying that FAM46C is a tumor suppressor in breast cancer and 2.2) defining which pathways are altered by the FAM46C complex in breast malignancies.

Expected results

We expect to determine which protein receptors or secreted cytokines regulated by FAM46C are important

for regulating specific immune cell functionality in the microenvironment (Aim 1.1). Moreover, among the pathways regulated by FAM46C, we will define which ones have a relevance in oncosuppression and will identify and test the targetability of selected enzymes (Aim 1.2). We also plan to establish a role for the FAM46C/FNDC3A complex in breast cancer (Aims 2.1 and 2.2).

Impact on cancer

We will identify therapeutical targets for MM treatment, establish FAM46C role in breast cancer and, consequently, define druggable targets also for treatment of breast malignancies. Our study will pave the road for studies on FAM46C functionality in cancers other than MM.

Federica Marasca **MFAG - 2023**

Fondazione "Istituto Nazionale Genetica Molecolare - INGM"

Reprogramming exhausted T cells through the disruption of chromatin condensates

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Background

Immune Checkpoints Inhibitors (ICIs) have symbolized an unprecedent progress in cancer therapy; however, most of the patients fail to achieve a successful response. One of the reasons is the establishment of a population of terminal exhausted T cell (Tex) that are insensible to ICIs treatment. Hence, the study of the epigenetic mechanisms steering the acquisition of a terminal exhausted phenotype is pivotal to design novel and more effective strategy to reprogram Tex into functional effector cells (Teff). At present, it is acknowledged that chromatin organizes into condensates to control gene expression; interestingly, defects in condensate assembly are associated to a plethora of diseases, representing an innovative window for drug discovery.

Here I present a solid bunch of evidence proving that in exhausted T cells heterochromatin is assembled in aberrant condensates, characterized by a marked increase of H3K9me3 foci. Interestingly, the interference with chromatin condensates drifts back Tex to a more functional phenotype.

Hypothesis

Here I postulate that T cell exhaustion is a "condensatopathy" as regulated by the aberrant assembly of heterochromatin condensates. I hypothesize that the interference with heterochromatin condensates could represents a new way to revert T cell exhaustion.

Aims

This proposal aims at illuminating how heterochromatin condensates steer terminal T cell exhaustion, a knowledge that is still missing in tumor infiltrating lymphocytes (TIL) biology. Furthermore, it aspires to demonstrate that targeting heterochromatin condensates in TIL could represent a novel, still yet unexplored frontier to define novel druggable molecules for cancer treatment.

Experimental design

I aim to study how heterochromatin condensates regulate the genome during the transition of Teff into precursor and terminal Tex. To this aim, I will define i) genes regulated by heterochromatin condensates by ChIP-seq for H3K9me3, Kap1 and HP1a and ii) their local proximity by Hi-ChIP for H3K9me3. Also, I will iii) inspect the topology of the identified heterochromatin condensates by DNA-FISH combined with H3K9me3 immunostaining in super resolution microscopy.

In parallel, I aim to identify which proteins compose and regulate the dysfunctional assembly of heterochromatin condensates in terminal Tex i) performing an optical CRISPR/Cas9 screening that tests H3K9me3 foci assembly by imaging. Finally, I plan to interfere with the assembly of the aberrant heterochromatin condensates to revert terminal T cell exhaustion ii) targeting the identified proteins with aptamers able to perturb the propensity of the molecules to phase separate. This approach has been designed to increasing the specificity of "epigenetic-based therapies", as it avoids to deplete the cells of

essential epigenetic regulators and/or enzymatic activities.

Expected results

This proposal promises to define new T-cell biology, discovering uncharted epigenetic mechanisms and to identify novel candidate targets and molecules for immunotherapeutic approaches in the promising field of "condensates-modifying therapeutics".

Impact on cancer

The produced results will go towards precision and increasingly specific medicine and could renovate immunotherapy approaches by targeting the "inner" of the cells instead of the "outer", namely awakening the effector response of TIL by drugging "chromatin condensates".

Ionica Masgras **MFAG - 2023**

Consiglio Nazionale delle Ricerche

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

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Background

The metabolic rheostat of tumor cells is often altered, thus posing a strong constraint in the tumor microenvironment and possibly shaping its immunological response. Despite a remarkable macrophage infiltration in malignant peripheral nerve sheath tumors (MPNSTs), Schwann cell (SC) cancers hardly hitting patients with Neurofibromatosis type 1 (NF1) genetic disease, the role of innate immunity is poorly understood. Tumor Associated macrophages (TAMs) are emerging as important players in the tumor microenvironment sustaining several pro-neoplastic and immunosuppressive activities. Recently, the diverse macrophage phenotypes have been found tightly connected to specific metabolic signatures pointing to the expression of certain metabolic enzymes as reliable markers of TAM specific functions. Narrowing down this information to key metabolic routes that sustain TAM pro-tumoral signals in MPNSTs could reveal novel therapeutic opportunities for reverting macrophage mis-behavior to the benefits of anti-neoplastic cures.

Hypothesis

Building on the metabolic adaptations discovered by our group and others in MPNST cells, we envisage that the metabolic status of tumor SCs, beside regulating tumor growth in a cell-autonomous manner, affects neighboring cells driving a pro-tumoral macrophage response. Uncovering specific TAM phenotypic/metabolic signature(s) in MPNSTs could bring space for novel anti-tumoral interventions based on macrophage re-education that could elicit an immune response effective in hampering MPNST growth.

Aims

The overall ambition of this proposal is to 1) Identify immune(metabolic) markers in MPNST samples from mouse models and human specimens, 2) Discover the metabolic-driven signals of MPNST cells that short-circuit pro-tumoral macrophage activities and 3) Re-educate the metabolism of tumoral macrophages to repress MPNST growth.

Experimental design

Exploiting our newly developed mouse model of MPNSTs we will characterize the phenotypic/metabolic features of macrophages infiltrating peripheral nerve neoplasms, thus defining a TAM metabolic signature. This will be subsequently tested in human MPNST specimens. We will combine transcriptomic and metabolomic analyses to pinpoint crucial metabolic routes through which tumor SCs signal to and engage neighboring macrophage metabolism for supporting cancer growth. A set of metabolic enzymes distinctive of MPNST-associated macrophages will be pharmacologically and genetically targeted assessing their anti-neoplastic efficacy in vitro and in vivo in immunocompetent MPNST-bearing mice.

Expected results

Merging macrophage markers of murine models and human MPNST specimens, we expect to identify key metabolic enzymes underlying TAM pro-tumoral functions in MPNSTs. Their targeting is expected to affect in vitro/vivo MPNST tumorigenicity and/or to improve the efficacy of immune checkpoint drugs, thus leading to translational applicability.

Impact on cancer

This proposal points to the identification and targeting of Achilles' heel in TAM metabolism to achieve anti-neoplastic goals against currently incurable MPNSTs. A successful outcome of these investigations will reveal not only unprecedented targetable actors in non-tumoral cells of MPNSTs but empower also the discovery of the paracrine signals released by MPNST cells to hijack macrophage behavior. Unraveling the immunological blockchains occurring in MPNSTs could amplify the number of targeted therapies available for these aggressive cancers creating possibilities for synergistic interventions.

Maria Giovanna Mastromarino **MFAG - 2024**

Azienda Ospedaliera Universitaria Pisana

Pressurized intra-thoracic aerosol chemotherapy in pleural carcinosis from lung cancer: in-vitro and clinical efficacy

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Background

Non-small-cell lung cancer (NSCLC) still represents the main cause of cancer mortality worldwide, often leading to pleural carcinomatosis (PC) and malignant pleural effusion (MPE) in about 20% of patients. In these cases, burdened by an abysmal prognosis, any intervention remains predominantly palliative to reduce clinical symptoms. Pleurodesis with sterile surgical talc via Video-Assisted Thoracoscopic Surgery (VATS) represents the standard treatment to prevent MPE recurrence. Pressurized Intra-Thoracic Aerosol Chemotherapy (PITAC) is a novel technique that could provide both pleurodesis and an antineoplastic effect by delivering chemotherapeutic aerosols directly into the thoracic cavity.

Hypothesis

PITAC uses a nebulizer to deliver chemotherapeutic drugs into the thorax during standard VATS, forming a therapeutic aerosol. The in-tissue drug influx is enhanced by generating a pressure gradient to overcome the tumour interstitial fluid pressure. Aerosol has a superior surface/volume ratio to liquids, and its higher drug concentration increases the in-tissue depth penetration. The advantage is the direct administration of chemotherapy on pleural sites of disease that would be difficult to reach by systemic therapy, and also allows uniform pleurodesis to control MPE recurrence.

Aims

The project aims to:

a) Validate PITAC as part of a new multimodal, loco-regional, tailored treatment for PC-NSCLC patients.

b) Develop an in-vitro model for pressurized aerosol chemotherapy using patient-derived cell cultures to evaluate the anticancer efficacy of PITAC drugs.

c) Assess the efficacy of different nebulized antineoplastic agents on patient-derived primary cancer cell cultures to determine the most effective drugs and optimal doses for treating PC-NSCLCs, with the prospective of a personalized cure.

Experimental design

The study population will be composed of patients affected by PC-NSCLC requiring a surgical operation to control MPE.

PITAC will be performed by VATS, delivering tailored doses of cisplatin and doxorubicin via a dedicated Nebulizer under a constant pressurization of thoracic cavity.

PITAC in-vivo efficacy will be assessed in terms of pleurodesis and local (intrapleural) disease control. The study of PITAC efficacy will be extended on an in-vitro model. Bi- and tri-dimensional patient-derived PC-NSCLC cell cultures will be established and treated in a dedicated experimental PITAC chamber model, to test different antitumor drugs, set the doses and evaluate the antineoplastic effect.

Expected results

We expect to obtain:

a) Effective pleurodesis both at 30-and 90-days after surgery as well as extended survival by improved local disease control with very low physical impairment and negligible systemic effects.

b) Increased antineoplastic effect of cisplatin and doxorubicin on vitality and proliferation of patient-derived PC-NSCLC cell cultures in PITAC in-vitro model, compared to standard conditions.

c) Validation of different drugs which could exert a more potent antineoplastic effect than cisplatin and doxorubicin on primary NSCLC cell cultures.

Impact on cancer

PITAC, as a new surgical method combined with more effective chemotherapy, could revolutionize locoregional treatment of PC-NSCLC, offering a tailored and less invasive approach. Significant clinical and socio-economic benefits include tailored therapies that maximize efficacy, reduce public health costs, minimize patient side effects, and improve survival rates and quality of life.

In summary, PITAC could potentially change PC-NSCLC natural history and emerging itself as a cornerstone of precision medicine.

Alessio Menga **MFAG - 2021**

Università degli Studi del Piemonte Orientale "Amedeo Avogadro"

Dissecting mitochondrial lysine and tryptophan metabolism to target metabolic symbiosis in lung adenocarcinoma

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Background

Lung adenocarcinoma (LUAD) is the most common histological type of lung cancer with mutations in EGFR and KRAS, translocation of ALK, and loss of LKB1 as the most frequent oncogenic alterations. These alterations have been shown to drive different metabolic programs of LUAD cells. Most of the studies focused on glucose and glutamine metabolism neglecting the role of alternative amino acids, such as tryptophan and lysine, in driving aggressiveness and immune escape. Despite the relevance of lysine and tryptophan metabolism for LUAD onset and progression has come to light, their role in supporting the mitochondrial bioenergetics and dynamics of LUAD cells remains elusive.

Hypothesis

Based on our preliminary results we identify the oxoadipate carrier (ODC), also known as SLC25A21 that drives the mitochondrial catabolism of tryptophan and lysine intermediates, as a key modulator of the malignant phenotype of lung cancer cells. ODC, through epigenetic mechanisms, sustains the expression of mismatch repair genes lowering the tumor mutational burden and the immunogenicity of the LUAD cells. Hence its targeting will rewire the crosstalk between cancer cells and immune cells.

Aims

This project combines my background in mitochondrial metabolism and cancer biology to identify: (i) the metabolic and epigenetic mechanisms by which ODC modulates the aggressiveness and the response to therapies of LUAD cells, (ii) the role of ODC-driven tryptophan and lysine metabolism in the establishment of an immune-tolerant microenvironment, (iii) ODC in human samples as a novel metabolic target correlated with LUAD progression, spread and immune evasion.

Experimental design

Through in vitro/vivo approaches, we will elucidate the mechanisms of metabolic adaptation, switching and alterations of immune and lung cancer cells in response to ODC modulation. In particular, genomic analysis techniques, NanoString Technologies, CIBERSORT-based deconvolution methods and validated bioinformatics analysis pipelines will be employed to unveil ODC-driven epigenetic mechanisms of immunoediting in LUAD. Furthermore human tissue microarrays will be used in order to disclose a possible correlation between ODC expression, oncogenic drivers, tumor invasiveness and immunogenicity.

Expected results

This study is expected to 1) decipher the metabolic and epigenetic consequences of ODC modulation in LUAD; 2) unravel the functional link with relevant oncogenic mutations; 3) unmask the role of ODC in the metabolic dynamics (competition and/or symbiosis) between cancer cells and their microenvironment and in immunotherapy response; 4) define ODC as a novel synthetic lethality and potential therapeutic target.

Impact on cancer

The outcome of this research might offer proof-of-concept that pharmacological inhibition of mitochondrial tryptophan and lysine catabolism can reduce cancer progression and improve the response to classical chemotherapy and/or advanced therapies (immune checkpoint inhibitors, KRASG12C inhibitors). ODC is just the tip of the "mitochondrial" iceberg where two essential amino acids degradation pathways converge. The deep diving into mitochondrial tryptophan and lysine metabolism could provide novel insights into the understanding of the metabolic adaptation mechanisms of LUAD disease and may pave the way for indentifying new metabolic targets to fight LUAD.

Caterina Miro

Università degli Studi di Napoli "Federico II"

Dissecting the effects of SIRT2 and SIRT6 modulation in cutaneous malignancy and chemoresistance.

Background

Sirtuins (SIRTs) are classified as class III histone deacetylases (HDACs), involved in several physio-pathological processes, including metabolism, inflammation and cancer.

SIRTs' dysregulation can be observed in many skin-related diseases, including psoriasis, melanoma, Squamous Cell Carcinoma (SCC) and Basal Cell Carcinoma (BCC). SIRTs have been reported in human tumors with a contradictory role, behaving as oncopromoters or oncosuppressors, depending on the SIRT and the cancer type. Our research group demonstrated that SIRT2 and 6 are up-regulated in the SCC chemical carcinogenesis mouse model and that their inactivation can delay SCC evolution. Besides regulating cancer biology, recent research has suggested that SIRTs can also be essential for the acquired resistance to chemotherapeutical agents.

Hypothesis

Based on our research findings, we hypothesize that the perturbation of the SIRT2 and 6 activities in SCC could produce an anti-proliferative effect and sensitize cancer cells to chemotherapeutics. Thus, our working hypothesis is that modulators of SIRTs activities can represent useful tools for developing new therapeutic strategies for unresectable SCC.

Aims

This project has three aims: in SA1 we will dissect the stage-specific expression and localization of SIRT2 and 6 in SCC cells and tissues. In vivo experiments in mouse models of SCC will enable us to unravel the role of these SIRTs in the multistep cancer process. In SA2 we will obtain a global view of SIRT2- and SIRT6-regulated genes in skin cancer. Furthermore, in SA3 we plan to analyse the effects of two new pharmacologic compounds able to selectively interfere with SIRT2 and SIRT6 activity. The use of SIRT2 and 6 inhibitors will provide a valid tool for the inhibition of SCC tumor growth and progression and will be exploited as a promising tool to overcome the cancer cells drug resistance.

Experimental design

In SA1, the impact of SIRT2 and 6 on SCC tumors will be evaluated using in vitro and in vivo approaches to determine the effects of their manipulation on SCC cell proliferation, migration and ability to form invasive tumors. Moreover, in vivo studies will be carried out using the SIRT2 (SIRT2fl/fl;K14Cre+, cSirt2KO) and SIRT6 (Sirt6fl/fl;K14cre+, cSirt6KO) conditional knockout mouse models. Upon induction of tumors by chemical carcinogenesis, the isolated SCC lesions will be used for high-throughput single-cell RNA-sequencing (scRNA-seq) to identify putative candidate SIRT2 and 6 targets (SA2). In SA3 we will study the ability of the pharmacologic inhibition of SIRT2 and 6 to mediate chemotherapy resistance.

Expected results

The combination of the proposed studies will enable us to explore the functional role of SIRT2 and 6 during the intricate multi-cascade process of epithelial cancers. Our final goal is to provide proof of concept of the use of pharmacological modulators of SIRT2 and 6 as therapy to impair tumor growth and drug resistance.

Impact on cancer

The specific epigenetic alterations leading to either slow-growing poorly invasive or more aggressive SCCs are still scarcely understood. By dissecting the function of SIRT2 and 6 in SCC tumors, we aim to identify new mechanisms and potential novel therapeutic targets for the treatment of cancers resistant to the currently available drugs.

Eugenio Morelli **Start-Up - 2023**

Istituto di Candiolo - Fondazione del Piemonte per l'Oncologia (FPO) - I.R.C.C.S.

Decoding the molecular pathways and therapeutic potential of long noncoding RNAs in multiple myeloma

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Background

Multiple myeloma (MM) is a malignancy of plasma cells that accounts for ~20% of deaths from hematologic cancers. More effective therapies are urgently awaited and may be informed by better defining the landscape of actionable tumor-promoting factors. Whereas most research focuses on proteins, there is emerging interest in the tumor-promoting activity of long non-protein coding RNAs (lncRNAs).

LncRNAs are key regulators of cellular function, acting either as gene regulatory elements or RNA transcripts. In this latter case, they are defined by having a length >200nt and lacking protein-coding potential. These RNA molecules outnumber protein-coding mRNAs in the human genome and are transcribed by dedicated promoters in a tissue-specific manner. A key function of lncRNAs is to provide molecular scaffolds for proteins and other nucleic acids. By this and other mechanisms, lncRNAs are becoming increasingly implicated in the progressive gain of a malignant phenotype by tumor cells.

In prior work, we identified a large number of differentially expressed lncRNAs in patient-derived MM cells versus healthy donor-derived plasma cells and found that lncRNAs are independent risk predictors for clinical outcome. We have started to functionally delineate the tumor-promoting activity of lncRNAs and demonstrated the actionability of two of them in animal models of MM (lnc-17-92 and MALAT1). Building on these early results, in this project we aim to better understand the tumor-promoting roles of lncRNAs and to unlock their therapeutic potential in MM.

Hypothesis

We hypothesize that lncRNAs control key survival pathways in MM cells and can be targeted for therapeutic benefit in MM-bearing animal models.

Aims

We will systematically define the tumor-promoting lncRNAs in MM and their clinical and functional features (AIM 1). Then, we will focus on a tumor-promoting lncRNA (RMRP) that has unique clinical, functional, and structural properties and that we hypothesize to be druggable for therapeutic effect. We will characterize its tumor-promoting mechanism (AIM 2) and develop small molecule and antisense inhibitors for its targeting in pre-clinical models of MM (AIM 3).

Experimental design

We will exploit the RNA-targeting endonuclease CRISPR-Cas13d to identify the tumor-promoting lncRNAs in an unbiased genome-wide manner and will couple it with single-cell RNA-seq (PERTURB-seq) to systematically define the transcriptional programs associated with the perturbations of the tumor-promoting lncRNAs (AIM 1). We will use RNA-protein interaction assays and live-cell microscopy to dissect the molecular interaction and functional role of a novel tumor-promoting lncRNA that we hypothesize provides an essential scaffold for the formation of nucleoli (AIM 2). Finally, we will optimize RNA-targeting

small molecule and antisense inhibitors of this lncRNA and test their anti-MM activity in cellular and animal models (AIM 3).

Expected results

We will define the tumor-promoting lncRNAs in MM and develop one of them as a therapeutic target with inhibitors for translation.

Impact on cancer

This project will unlock a novel repertoire of actionable targets and develop an effective way to target them. Our data will be a key asset to the MM and lncRNA fields and accessible on a public portal.

Kiu Yan Charlotte Ng **Start-Up - 2024**

Università Humanitas

An artificial intelligence platform to predict cancer patient outcomes from multi-omic data

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Background

Artificial intelligence (AI) is revolutionizing oncology by leveraging deep learning techniques for enhanced image processing in pathology and radiology. The integration of AI with multi-omics data-comprising genomics, transcriptomics, proteomics, and metabolomics-promises a comprehensive understanding of cancer heterogeneity, essential for personalized oncology. However, appropriate modelling of the biological interdependence among omics layers, the handling of missing data and data scarcity remain significant challenges to wider adoption of AI multi-omics models in oncology.

Hypothesis

The integration of multi-omics data using AI models can yield robust prognostic biomarkers, enhancing the precision of cancer diagnosis and treatment. We hypothesise that i) incorporating cross-omics learnings can overcome challenges related to the biological interdependence among omics layers, ii) data augmentation and improved AI models can help with missing data and iii) the incorporation of biological insights from complementary omics assays provide added knowledge to overcome data scarcity.

Aims

1. Address challenges of omics interdependence, missing data and data scarcity in the development of multi-omics AI models in oncology
2. Develop a multi-omics AI framework for the identification of prognostic biomarkers
3. Apply these models to hepatocellular carcinoma (HCC) to identify prognostic biomarkers and improve patient stratification

Experimental design

WP1. Data Augmentation: For our existing multi-omics dataset of 122 HCCs, augment the (phospho)proteome data using neural networks and develop models for cellular classification from H&E-stained slides to extract detailed histopathological features.

WP2: Inference of gene regulatory mechanisms in HCC: Perform single-cell RNA+ATAC-seq on 30 HCCs and 10 normal liver samples to construct cell type-specific gene regulatory networks (GRNs).

WP3. Identification of tumor effectors from CRISPR screens: Optimise statistical frameworks to identify HCC effectors from CRISPR screen data.

WP4. Prognostic model development: Develop machine learning (ML) models integrating multi-omics and pathology data to identify prognostic biomarkers, incorporating learnings from GRNs and tumor effectors.

WP5: Validation of biomarkers: Validate biomarkers from the prognostic models in an external cohort.

Expected results

1. Enriched multi-omics datasets with inferred (phospho)proteome profiles and digital pathology.

2. Comprehensive single-cell RNA+ATAC-seq data, revealing cell type-specific GRNs in HCC.
3. Optimized statistical framework for CRISPR screens, identifying HCC-specific effectors.
4. Robust ML models providing prognostic biomarkers validated in independent cohorts.

Impact on cancer

This project aims to advance AI-driven precision oncology by integrating multi-omics data and computational pathology. The development of robust prognostic models will improve diagnostic accuracy and treatment personalization in HCC, potentially serving as a blueprint for other cancer types. These advancements promise to enhance patient care by enabling more informed clinical decisions and improving overall outcomes in oncology.

Lara Paracchini

Università Humanitas

Diagnosis of tubal malignant ovarian cancer precursor lesion through the analysis of DNA from Pap test smear

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Background

A significant number of women with germline pathogenic variants in BRCA genes (gBRCA) have a high risk of developing High-Grade Serous Epithelial Ovarian Cancer (HGS-EOC), with a 40% risk for women with a BRCA1 mutation and a 20% risk for those with a BRCA2 mutation by age 80. Risk-reducing surgery (salpingo-oophorectomy) is universally recommended from the age of 35 for BRCA1 heterozygotes and from the age of 40 for BRCA2 heterozygotes. The pathological analysis of the fallopian tubes of women undertaking prophylactic surgery, shows early neoplastic lesion known as serous tubal intraepithelial carcinomas (STICs) in only 5% of cases. Unfortunately, there are no sensitive and non-invasive methods to detect STIC, making it difficult to determine when prophylactic surgery should be performed. We have recently shown that HGS-EOC can be detected up to 9 years before diagnosis by examining the genomic instability profile (Copy number Profile Abnormality, CPA) of DNA purified from Pap test smears. The sensitivity of our test was 75% and the specificity 96%, suggesting the potential use of CPA score for early detection of HGS-EOC (Paracchini et al., Science Translational Medicine 2023). The analysis of other variables (methylation status, type of BRCA variants and clinical features) might further increase the predictive accuracy our test.

Hypothesis

We hypothesize that the overall genomic instability (CPA score) of DNA obtained from cervico-vaginal swabs is a sufficiently sensitive and specific biomarker to detect the presence of STIC/in situ carcinoma. We hypothesize that integrating epigenomic (DNA methylation), genetic (type of gBRCA mutation) and demographic/clinical features -using machine learning algorithms- a further improvement of the specificity and sensitivity of our test can be achieved.

Aims

i) To evaluate if the CPA score can serve as a surrogate biomarker for early-stage malignancy, thus improving surveillance and indicating the optimal time for prophylactic surgery in gBRCA women. ii) To assess if the predictive accuracy can be increased by multimodal approach optimized by machine learning algorithms.

Experimental design

We plan to analyze tubal tissues and matched Pap test samples from at least 260 gBRCA women who have undergone prophylactic surgery. Pathological examination will assess the presence of STIC/in situ carcinoma, while shallow whole genome sequencing will allow to determine the CPA score. The concordance between the results of the pathological analysis and of the molecular assay will be evaluated. On the same samples, the methylation profile of Pap test DNA in specific genomic regions will be assessed using methylation sensitive restriction enzymes and droplet digital PCR. Results derived from CPA and epigenomic analysis will be integrated with the type of BRCA variants and demographic/clinical features to derive a multimodal risk score using machine learning algorithms.

Expected results

The development of a specific and sensitive risk score to predict the presence of STIC/in situ carcinoma in gBRCA women undergoing prophylactic surgery.

Impact on cancer

The development of a reliable and non-invasive screening test for the early detection of HGS-EOC will significantly improve the clinical management of gBRCA women, by defining the need and timing of prophylactic intervention.

Monica Parodi **MFAG - 2024**

Ospedale Policlinico San Martino - IRCCS per l'Oncologia

Synergy of Natural Killer cells and anti-osteolytic drugs in the prevention and treatment of bone metastasis.

Background

Metastatic cancer represents a complex disease caused by a series of simultaneous and partially overlapping events. Nowadays, the mechanism of metastasis development has not yet been entirely understood. Even more importantly, the therapeutic control of metastatic progression is far to be achieved. In particular, bone represents a main site for the metastatic spread in different tumor types including breast, prostate and lung.

Hypothesis

NK cells represent powerful immune effectors to contrast tumor development. Different strategies are being investigated to optimize NK cell efficacy in different tumor settings. Enhancing NK cell activity to target bone metastases combined anti-osteolytic drugs represent a potential therapeutic synergy.

Aims

AIM1: Development of a 3D in vitro bone metastasis model and the evaluation of the impact of metastasis development on the bone marrow (BM).
AIM2: Determine the therapeutic potential of NK cells alone or in combination with drugs in bone metastasis development in vitro.
AIM3: Validation of the results obtained in 3D in vitro model in in vivo Human-in-mice model.

Experimental design

We will study:
1) The set-up of 3D in vitro bone metastasis model using fresh bone from hip arthroprothesis and tumor cell lines derived from breast, prostate and lung.
2) Functional and phenotypical characteristics of BM cell subsets after bone metastasis development.
3) The cross-talk between NK cell populations and bone metastasis microenvironment.
4) The ability of anti-osteolytic drugs to enhance the effect of NK cells.
5) The efficacy of appropriate NK cell/drug combinations in the mouse model, to validate the data obtained in vitro.

Expected results

We expect to achieve the following results:
1) Development of a 3D in vitro bone metastasis model suitable for a wide range of preclinical studies.
2)Characterization of the changes of BM cell populations possible occurring during metastasis development, and identification of possible therapeutic targets.
3) Definition of the NK cell populations able to better counteract spread and development of bone metastases.
4) Identification of possible effective anti-osteolytic drugs maximizing the NK-based immunotherapy against

metastatic disease.

Impact on cancer

The study of the cross-talk between NK cells and metastatic BM will provide possible new targets which could be useful to conceive effective therapeutic strategies. The possible effect of anti-osteolytic drugs in supporting NK cell activity will represent a further tool to get therapeutic synergies, enhancing efficacy and reducing the impact of side effects.

Sandro Pasquali **Next Gen Clinician Scientist - 2023**

Fondazione I.R.C.C.S. Istituto Nazionale dei Tumori - Milano

Dissecting immunological effects of neoadjuvant therapies in primary high-risk soft tissue sarcomas

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Background

Patients with high-risk soft tissue sarcomas (STS) at low survival probabilities are treated with neoadjuvant anthracycline and ifosfamide (AI) to lower their risk of developing metastatic disease after surgery. However, roughly one in two patients eventually recur mostly at distant site with limited therapeutic opportunities.

Hypothesis

Changes to immune infiltrate after neoadjuvant chemotherapy may effectively result in an immune induction that sensitizes tumours to an immune checkpoint inhibitor (ICI).

Aims

This study proposal is meant to demonstrate an immune modulation in primary localized high-risk STS of extremity or trunk wall after neoadjuvant chemotherapy with AI. Ultimately, these findings will provide evidence for a rationally designed and feasible randomised clinical trial (RCT) that will investigate an ICI in these patients. The amount of data generated to reach this main aim of this study will be exploited to identify whether an in-depth characterisation of the immune infiltrate could enhance the prognostic accuracy of patient risk stratification performed with the nomogram Sarculator, a widely diffused prognostic tool for STS.

Experimental design

Firstly, we will deeply investigate tumour immune infiltrate in samples of high-risk STS with the five most common histologies of extremity or trunk wall (undifferentiated pleomorphic sarcoma, synovial sarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumour, myxoid liposarcoma) obtained before and after neoadjuvant AI. We will exploit multiple approaches, including the search for tertiary lymphoid structures (TLS), multiplexed IHC, and spatial transcriptomic analysis (WP-1). In order to monitor markers of tumour response and immunomodulatory effects of neoadjuvant AI at the systemic level, we will collect patient peripheral blood at three time points (i.e., diagnosis, after AI, after surgery) and test the presence of circulating tumour DNA (ctDNA) as well as the frequency and activation of myeloid, T-cells and B-cells (WP-2). All these data will be exploited to enhance the accuracy of Sarculator (WP-3). In parallel, we will validate a prognostic score, which was previously generated and is part of the preliminary data that support this study proposal. To validate findings from WP-1 and test effectiveness of ICI following neoadjuvant AI we will exploit patient-derived explants (PDE) of treated and untreated tumour samples (WP-4). Finally, we will rationally design a RCT for high-risk STS to be conducted within the Italian Sarcoma Group, the Italian network for sarcoma research and treatment (WP-5).

Expected results

We expect to identify changes induced by neoadjuvant AI in tumour cells, tumour-infiltrating and circulating immune cells in the most common high-risk STS of extremity or trunk wall. We also expect to improve

current tools for patient staging.

Impact on cancer

This study will impact the field of cancer in five year times through generating compelling preclinical evidence through profiling immune modulation of AI in patients with selected high-risk STS both at the tumour and systemic levels.

Simone Patergnani

Università degli Studi di Ferrara

Relevance of mitochondrial HMGB1 for malignant pleural mesothelioma: from the neoplastic transformation to the therapy

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Background

Pleural mesothelioma (PM) is an aggressive cancer highly correlated with exposure to asbestos. Once inhaled asbestos provokes a chronic damage to the mesothelial surface, causing malignant transformation. However, it remains unknown the precise mechanism(s) by which asbestos drives the initiation of the cell transformation process.

A deeper understanding of the molecular means involved in the onset and progression of PM is imperative to unveil targets for new therapeutic strategies.

Hypothesis

Several discoveries suggest that high mobility group box-1 (HMGB1) is a critical mediator of asbestos-induced PM initiation.

However, the HMGB1-related mechanisms regulating the malignant transformation are still unknown.

We hypothesize that, during the asbestos-induced cell transformation, HMGB1 is released from the nucleus to localize on the mitochondrial surface.

Here, HMGB1 induces mitochondrial dysfunctions and variations in ferritinophagy (a particular form of autophagy important for several cellular functions) and exacerbates the inflammatory environment, through the regulation of inflammasomes.

Aims

AIM.1: Investigate how dynamic changes of HMGB1 provoke mitochondrial dysfunctions and alter the iron-related mechanisms to regulate the chronic inflammatory state necessary to drive the malignant transformation.

AIM.2: Demonstrate that the asbestos-driven transformation is dependent on a mitochondrial localization of HMGB1.

AIM.3: Enforcing the anti-neoplastic properties of PM chemotherapeutic agents through strategies targeting the harmful HMGB1-mediated events.

Experimental design

The intracellular localization of HMGB1 as well as elements of the mitochondrial functioning, ferritinophagy and inflammasome will be monitored during the malignant transformation of primary human mesothelial cells and in mice exposed to asbestos using intrapleural injection. Variations of these intracellular dynamics will be also measured in tissue samples obtained from PM patients. Next, a series of HMGB1 chimeras inducing HMGB1 expression in different intracellular compartments will be designed and transduced in primary cultures obtained from conditional HMGB1 knockout mice to clarify the localization of HMGB1 that is important for exert these effects. Finally, the most promising compounds modulating the harmful HMGB1-mediated events identified in vitro will be tested in two murine models of PM to demonstrate that by

targeting these molecular pathways it is possible to counteract the PM progression and recurrence.

Expected results

We plan to unveil new molecular mechanisms of the involvement of HMGB1 in the evolution of the PM, in particular a specific molecular liaison composed of mitochondria, ferritinophagy and inflammasome. Further, the project will permit to understand whether monitoring HMGB1-related molecules may represent new tools to improve the diagnosis.

Finally, the project aims to reveal novel molecular strategies to improve the efficacy of conventional chemotherapy against PM.

Impact on cancer

PM is an incurable form of cancer characterized by a median survival from diagnosis of only 1 year. No effective therapies exist for this devasting disease.

Furthermore, PM is characterized by a rapid recurrence after surgery, as it is impossible to remove and kill every cancer cell.

This proposal aims to dissect new molecular mechanisms which occur during the malignant transformation of a mesothelial cells.

We are confident that a successful completion of this project will provide critical information for development of future therapies for PM and (in future) other cancers.

Ilenia Pellarin

Centro di Riferimento Oncologico - Aviano - I.R.C.C.S.

Unveiling the role of the splicing factor p54nrb in Epithelial Ovarian Cancer dissemination and chemoresistance

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Background

In Epithelial Ovarian Cancer (EOC) platinum (PT)-based chemotherapy represents the backbone of the first line treatment, but it is frequently followed by resistant recurrences. PT-resistant disease, is often paralled by PARP inhibitors (PARPi) acquired resistance, suggesting the coexistence of overlapping resistance mechanisms. Changes in RNA splicing regulators and alternative splicing (AS) patterns, were observed in association with acquired chemoresistance or under the selective pressure of PT-treatment, in several human tumours. We have recently identified the splicing regulator complex SFPQ/p54nrb as a key factor in the modulation of PT sensitivity in EOC, linking the DNA damage response (DDR) to apoptosis. Our preliminary data suggest that p54nrb silencing reduces EOC cell survival and growth in vitro and in vivo. Interestingly we also found that p54nrb silencing, by increasing the DDR, influences not only the response to PT but also seems to synergize with PARPi.

Hypothesis

Increasing evidences suggest that AS plays a key role in cancer progression and that targeting the splicing factors activity in cancer is a feasible strategy. We hypothesize that p54nrb is a key factor in the regulation and interplay of AS, DNA damage and chemoresistance in EOC.

Aims

Our main goals are to dissect the molecular mechanisms by which p54nrb is regulated under PT-treatment and how these processes could affect cell survival, proliferation and chemoresistance. We aim to improve our knowledge on PT-resistance, proposing new therapeutic strategies. Based on our preliminary results, targeting p54nrb could represent a novel and promising approach to improve PT-effectiveness, possibly overcoming untreatable chemoresistance appearance in EOC.

Experimental design

To achieve our aims the research plan entails 4 interconnected but conceptually self-standing Work Packages.The project will be carried out using a combination of in vitro functional assays in different cellular models (modified for p54nrb expression) and in vivo approaches. WP1 will clarify how p54nrb is involved in the regulation of dissemination capabilities in EOC. Then we will evaluate how p54nrb can impact on different components of the tumour microenvironment. In WP2 we will dissect the mechanisms underlying the regulation of p54nrb under PT-pressure and how this could affect p54nrb interactome (proteomic approach).WP3 will be dedicated to understand how p54nrb mechanistically influences the response to chemotherapy: 1) estimating how PT can modify p54nrb affinity to RNA (RNA-seq/RIP-seq) 2) dissecting p54nrb function in the interplay with DDR (PT/PARPi response). WP4 will estimate the putative predictive/prognostic role of p54nrb in EOC, exploring p54nrb expression in clinical samples available in our Institute, and from collaborators, to understand its translational impact.

Expected results

We expect to clarify mechanistically the role of the multi-scaffold protein p54nrb and how its activity could influence DDR and response to therapies in EOCs. We expect that targeting p54nrb could represent an alternative and more specific way to improve PT-effectiveness, especially in PT-resistant patients.

Impact on cancer

We consider that this project will directly and positively impact on the management of EOC patients, by identifying novel biomarker of therapy resistance and/or by proposing new therapeutic strategies based on the targeting of RNA splicing.

Livia Perfetto **MFAG - 2023**

Università degli Studi di Roma "La Sapienza"

Modelling cell communication in Pancreatic Cancer: A Systems Biology Approach to Personalized Treatments

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Background

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a tumor microenvironment where cancer-associated fibroblast (CAFs) and cancer cells cross-talk via signals that result from the activation/repression of cell-specific signaling cascades. These processes sustain tumor growth and result in the deposition of a dense fibrotic stroma that shields the tumor from therapeutic treatments. Patient-specific proteogenomic profiling of cancer biopsies have shed light on general molecular events underlying PDAC tumorigenesis and progression, but have failed to clarify the molecular mechanisms that are deregulated in individual patients.

Hypothesis

The premise behind this proposal is that, by integrating all the molecular evidence collected over recent years, it should be possible to computationally model the dynamics of the events underlying the cell cross-talks occurring during PDAC onset and development at patient-resolution level. This step is necessary toward the development of effective personalized treatments. I advance that networks of causal interactions assembled by collating literature-evidence can provide a functional framework to integrate and interpret multi-omics datasets and build patient-specific models that are mechanistic or predictive.

Aims

This proposal aims at modeling the impact of cell communication on PDAC tumorigenesis and cancer progression to accelerate the rational usage of proteogenomic data in clinical practice. Specifically, I aim to i) Provide understanding of the molecular mechanisms underlying microenvironment formation; ii) Identify prognostic biomarkers; iii) Contribute to accelerate the identification of personalized therapeutic targets; iV) Support clinical decision-making.

Experimental design

First, I will annotate literature-derived interactions that play a role in the mentioned processes. Next, I will exploit a tool that I recently developed (SignalingProfiler) to combine curated data with publicly-available proteogenomic profiling of four large cohorts of PDAC samples to derive patient-specific mechanistic models of these processes. I will use them to derive biomarkers and prognostic factors (by applying machine learning followed by in silico and ex vivo validation); and to build patient-specific multi-scale models of intra- and intercellular communication between CAFs and cancer cells. These models will be trained with public data and tested to predict novel druggable nodes whose modulation can revert the malignant phenotype. Finally, I will develop a resource to access the project results.

Expected results

The main deliverables of this proposal are: i) a comprehensive description of interactions underlying PDAC; ii) 500 patient-specific mechanistic models; iii) a panel of biomarkers and prognostic factors; iv) approx. 30

patient-specific multi-scale models of intra- and intercellular communication between CAFs and cancer cells; v) a ranked list of patient-specific targettable nodes; vi) a publicly-available resource for a rationale usage of proteogenomic data in clinical practice.

Impact on cancer

With a 5-year survival rate below 10%, PDAC remains one of the deadliest cancers. The development of novel and effective treatments, relies on the dissection of the cross-talk that sustains and protects the tumor. My proposal aims at addressing this point by delivering computational tools to model the tumor development and response to genetic and chemical perturbations. The project is innovative and, if successful, will have an impact in our understanding of PDAC etiology, in our ability to make accurate prognosis and in the design of effective treatments.

Gianluca Petris **MFAG - 2024**

Fondazione Italiana Fegato - O.N.L.U.S.

Isogenic chromosome transfer: a synthetic genomics tool for modelling aneuploidy and cancer evolution

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Background

Aneuploidy and micronuclei are hallmarks of cancer and are implicated in genome instability and ecDNA formation. Loss of heterozygosity (LOH), including copy neutral LOH (CN-LOH) and gain LOH (G-LOH), is another class of common cancer genomic aberrations. There is essentially a void of isogenic and universally reproducible methods to model these critical cancer genomic events. For synthetic genomics purposes, I have developed a toolbox for chromosome transfer and elimination (transplant) suitable for creating any specific aneuploidy, micronuclei, and LOH events in somatic and stem cells.

Hypothesis

This proposal is built on the hypothesis that my chromosome transplant genome engineering toolbox offers an ideal and transformative methodological platform to model in any cell type, any aneuploidy and LOH events in a reproducible and controllable manner. Coupling specific isogenic chromosome-scale genome engineering with modulation of cellular genes, this project will deliver mechanistic insights into tumorigenesis, facilitate understanding and predicting tumour evolution and lead to the identification of novel cancer vulnerabilities.

Aims

I will apply chromosome-scale genome engineering as a broadly applicable, universal toolbox for modelling, understanding and finding genetic vulnerabilities of cancer. I will 1) generate isogenic cancer aneuploidy and G/CN-LOH models by leveraging on isogenic chromosome transplant technologies, and 2) characterise the role of genes altering chromosomes stability in micronuclei and discover novel aneuploid specific cancer vulnerabilities, which 3) will be mechanistically validated in 3D models and in vivo.

Experimental design

I will use two methods for chromosome transfer and chromosome specific elimination approaches, to generate specific aneuploidy and LOH in cells and organoids suitable for in vivo modelling in NSG mice. Resulting models will be validated by multi-omics analysis. I will take advantage of CRISPR knock-out and CRISPRa screenings in cells and organoids to identify and validate genes involved in genome/chromosome stability, aneuploid cancer cell survival and therapy resistance.

Expected results

This research will produce reliable, chromosome-scale, isogenic cells and organoids with chromosome-specific aneuploidy and LOH to study cancer, resulting in a thoroughly validated universal technological platform for chromosome-scale cancer cell genome engineering. I will use the resulting models as innovative screening platforms for the identification of factors and mechanisms regulating the impact of specific aneuploidy and LOH events, enabling to discover genetic and pharmacological targets to control and

modulate cancer cell survival.

Impact on cancer

The validation of the new largely applicable toolbox to engineer the genome at chromosome-scale will significantly broaden the set of technologies and experimental systems available to study the impact of large-scale chromosomal alteration in cancer and it will pave the way for future cancer studies. This project will also enable to expand our understanding of the complex genomic events driving carcinogenesis and will identify aneuploid cancer specific vulnerabilities for the development of sustainable and personalised medicinal approaches.

Sara Pilotto **Next Gen Clinician Scientist - 2024**

Università degli Studi di Verona

A perSonalized nuTrition and physicAl exeRcise intervention to enhance early-stage NSCLC ouTcomes: the STARLighT study.

Background

A healthier lifestyle (not smoking, maintaining a healthy weight, exercising regularly) could have avoided >1,600,000 lung-cancer-related deaths in 2019. Lifestyle interventions positively impact on lung cancer natural history across all disease stages. However, solid clinical evidence of the impact of lifestyle interventions on hard clinical endpoints (e.g., cure rates) in early-stage disease is currently lacking.

Hypothesis

STARLighT builds on the hypothesis that implementing a structured lifestyle intervention together with systemic neoadjuvant/adjuvant treatment would significantly improve clinical outcomes in patients with early-stage lung cancer and reduce the socio-economic burden caused by this disease, while substantially improving patients' quality-of-life.

Aims

The primary aim of the STARLighT trial is to test the impact of a structured and evidence-based lifestyle intervention delivered by the user-friendly portable interface (e-ALLY) on pathological complete response (pCR) in patients undergoing neoadjuvant treatments and on disease-free survival (DFS) in patients undergoing adjuvant therapies. Translational studies will allow understanding the biological and molecular mechanisms of action of lifestyle interventions, thereby generating novel biological knowledge and leading to progressive refinement of intervention strategies.

Experimental design

A master clinical protocol will be set up to evaluate e-ALLY assisted lifestyle intervention in different cohorts of patients diagnosed with non-oncogene addicted with early-stage NSCLC (stages IB to IIIA). COHORT A will accrue 35 patients undergoing neoadjuvant chemo/immunotherapy and exploit a single-arm phase II design to detect an increase in the proportion of pathological complete response (pCR) from 20% to 40%; COHORT B will accrue 216 patients undergoing physician's choice of adjuvant treatment and exploit a 2:1 randomized controlled design to detect an increase in 2-year disease-free survival (DFS) rates from 55% to 70% in the control and experimental arms, respectively. All patients in COHORT A and patients randomized to the experimental arm in COHORT B will receive a tailored, evidence-based lifestyle intervention (physical exercise, nutritional, behavioral support), based on the treatment setting and delivered through a telehealth system (e-ALLY), developed within the current project; patients allocated to the control arm (COHORT B) will receive the current standard of care and dedicated educational health material. Translational studies will encompass baseline assessment of genomic and transcriptomic features and longitudinal assessment of circulating immuno-inflammatory profiles and tumor microenvironment composition, to be correlated with clinical and lifestyle data, to build a comprehensive predictive model and to refine the e-ALLY-based intervention.

Expected results

We expect that a tailored lifestyle intervention will result in a higher pCR rate and longer DFS in patients in the neoadjuvant or adjuvant setting, respectively, thereby potentially increasing cure rates in early NSCLC. New biological knowledge is expected to be generated by translational studies, paving the way for even more effective future lifestyle-based interventions.

Impact on cancer

The ambitious aim of STARLighT is to provide solid evidence for affordable, easily implementable, patient-centered lifestyle interventions to reduce lung cancer mortality. Not secondarily, we expect such interventions to result in improved efficacy of current treatments, better patients' quality of life, and improved physical and psychological well-being of lung cancer survivors.

Valentina Proserpio **MFAG - 2023**

Università degli Studi di Torino

Dissecting the molecular basis of Women's Extramammary Paget Disease malignancy by high-resolution omics

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Background

Extramammary Paget Disease (EMPD) is a rare malignant intra-epidermal adenocarcinoma that displays a high recurrence rate following surgical excision (40%) and is often accompanied by underlying malignancy (10%-30%). If surgery is not an option, for invasive EMPD (~10%) the outcomes are generally poor for the lack of curative therapy. Data about female vulvar PD (VPD) are almost absent, because of the rarity of the disease itself, but also due to a strong male predominance in the Asian population (where most of the studies are conducted). Surgery is often extensive and includes total vulvectomy, thus severely impairing the patient's quality of life.

Hypothesis

The molecular knowledge on EMPD comes from studies conducted on almost exclusively male patients. To fill this gap and understand if the same or other deregulated cellular and molecular mechanisms are present, we will investigate the molecular and cellular basis of VPD in a unique collection of female VPD samples including non-invasive and invasive, recurrent and not recurrent, Imiquimod responders and not-responders. Being a rare disease, this analysis represents a crucial resource to investigate the molecular basis of invasion, recurrence and Imiquimod responsiveness in women. The most promising/deregulated mechanisms will be then functionally validated first in vitro and then in vivo, to identify new potential therapeutic targets and prognostic markers in invasive VPD.

Aims

This project aims at increasing our understanding of VPD in women at molecular and cellular level to identify new deregulated genes or pathways to improve diagnosis, monitoring and therapy of VPD. This project aims at the identification of new patients stratification strategies to avoid extensive and mutilating surgeries, whenever are therapies are likely to be efficient.

Experimental design

Perform RNA-seq on FFPE/fresh tumor and healthy matched skin samples. From freshly resected samples we will perform Single-Cell RNA-seq and we will generate stable cell lines from both cancer cells and healthy surrounding tissues. These cells will be instrumental for perturbation and validation experiments to identify deregulated pathways and genes in vitro. Best hits will be then validated in vivo.

Expected results

With this project we aim at the identification of new biomarkers for detecting patients at risk of recurrence and new biomarkers to divide patients in subgroups for selecting the best intervention therapy in individual patients (personalized medicine).

Moreover, we aim at the identification of putative new therapeutic targets for re-purposing studies, thus

reducing the surgical, often mutilating, approach.

Impact on cancer

Patients with VPD typically require extensive and often multiple surgeries due to its high recurrence rate. Moreover, the surgical treatment results in mutilating resections, with considerable morbidity, substantially affecting the patients' quality of life. Therefore, the development of new treatment for VPD and the identification of stratification strategies are both extremely significant and of great interest. Filling the gap in knowledge about VPD and defining the molecular mechanisms at the basis of VPD will help the clinicians in directing the therapies to limit the surgical approach.

Soraya Puglisi

Università degli Studi di Torino

Impact of Stress-Induced Steroid Secretion on outcome of Immunotherapy in patients with lung cancer: the SISSI study

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Background

Immune checkpoint inhibitors (ICIs) have changed the outcome of patients with different cancers. However, their benefits are not universal and predictive factors of response are still to be clearly outlined. Studies showed that the use of glucocorticoids (GCs) could limit ICI efficacy due to the immune suppressive action of GCs. Since GCs are "stress hormones", it is conceivable that the hypothalamic-pituitary-adrenal (HPA) axis is triggered in patients with advanced cancer, who live in a condition of chronic stress. Preliminary data suggest: i) an association between the disturbance of the HPA axis and earlier mortality; ii) improvement of the alterations of HPA axis following mindfulness-based stress reduction (MBSR), a group-based intervention program to relieve stress. However, no study has investigated the HPA axis in patients with immunotherapy-treated cancers.

Hypothesis

In patients with immunotherapy-treated lung cancers:
- the disturbance of the HPA axis, with consequent elevation in stress GC levels, is frequent
- this disturbance may cause immunosuppression thus impairing response to ICIs.
- abnormalities in the HPA axis may be associated with survival outcomes
- MBSR can improve the disturbance of the HPA axis.

Aims

'- To describe the type and frequency of the abnormalities of the HPA axis in patients with immunotherapy-treated lung cancers.
- To explore the correlation between these abnormalities and response to ICIs in terms of survival.
- To evaluate changes in the HPA axis using MBSR.

Experimental design

The SISSI study consists of two tasks, both including patients with non-small cell lung cancer (NSCLC) treated with ICIs:

- TASK 1 (months 4-56): assessment of the HPA axis and survival outcomes in an observational cohort (100 patients);
- TASK 2 (months 16-56): to evaluate the effect of MBSR on the HPA axis; 5 groups of 13 patients (interventional cohort) will undergo an 8-week period of MBSR (weekly sessions including a standard set of mental and physical mindfulness exercises).

Hormonal assessment (salivary and blood samples for steroid profiling) and psychological measures [Depression Anxiety Stress Scales - DASS-21; quality of life questionnaire (EORTC-QLQ-C30)] will be performed at scheduled timepoints (for TASK 1: pre-treatment and 6-month post-treatment with ICIs; for TASK 2: at the start and at the end of the 8-week MSBR, then every 6 months after MBSR).

Expected results

- High frequency of abnormalities of the HPA axis and association with worst scores in the questionnaires
- Greater abnormalities in the HPA axis and higher stress GC levels in patients with worst survival.
- Reduction in stress GC levels and in questionnaires after MBSR.

Impact on cancer

- To extend our knowledge on the endocrine alterations induced by chronic stress and their potential effect on quality of life and life expectancy, fueling future clinical intervention studies.
- To assess the reversibility of psychological stress and associated endocrine alteration by MBSR programs, that could become part of the management of patients on immunotherapy as a non-pharmacological strategy to improve psychological symptoms.
- Provide data for designing a future trial to investigate the potential effect of MBSR programs on survival of immunotherapy-treated patients.

Gian Luca Rampioni Vinciguerra **Start-Up - 2024**

Università degli Studi di Roma "La Sapienza"

Fra2 and cell stress response: exploring novel mechanisms of progression and therapeutic approaches in pancreatic cancer

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Background

Pancreatic ductal adenocarcinoma (PDAC) is notoriously resistant to treatment, with its aggressive nature heavily influenced by the unique tumor microenvironment (TME). Characterized by various non-cancer cells and a dense stroma, the TME limits oxygen and nutrient supply. As a result, PDAC cells must continuously reprogram their transcriptome to survive and evolve in this challenging milieu, adapting to diverse stresses and therapeutic pressure. Fos-related antigen-2 (Fra2) is a transcription factor, rapidly activated by several stimuli, including stress. Fra2 is frequently dysregulated in cancer and its consensus sequence is one of the most accessible in the PDAC chromatin. Recent literature evidence and preliminary data point to Fra2 as a critical modulator of gene expression in PDAC, conferring plasticity and resistance to metabolic stress, hypoxia, immune response, and therapy with KRAS inhibitors.

Hypothesis

Our overarching hypothesis posits that Fra2 exerts a multifaceted influence on PDAC, orchestrating transcriptional reprogramming in response to stress and microenvironmental cues. Fra2 activity not only nurtures the functional heterogeneity commonly observed in PDAC but also fuels the insurgence of therapeutic resistance and interplays with the TME, thereby shaping the trajectory of cancer cell evolution.

Aims

To address the knowledge gap surrounding the Fra2 role in PDAC, we aim to: 1) Elucidate the molecular mechanisms underpinning the stress-tolerant phenotype; 2) Explore the Fra2 involvement in the crosstalk between cancer and non-cancer cells within various microenvironmental niches throughout PDAC progression; 3) Characterize the Fra2-driven molecular mechanisms responsible for the resistance to KRAS-inhibitors; 4) Develop a selective Fra2-inhibitor for innovative combination therapies.

Experimental design

This proposal entails 4 conceptually self-standing Work Packages, leveraging integrated approaches and innovative technologies. WP1 will utilize an in vitro reversible Fra2-knockdown model to monitor PDAC response to various stresses (namely oxidative, mechanical, hypoxic, and metabolic stress). An integrated multi-omic approach will enable us to investigate the mechanisms of PDAC adaptivity. In WP2, we will establish a Fra2KO transgenic mouse model of PDAC to elucidate the Fra2 role in the dynamic interplay with the TME throughout tumor evolution. We will analyze the transcriptome of spatially annotated neoplastic niches in relation to TME structures and communities. Utilizing patient-derived organoids, WP3 will investigate the KRAS-inhibitor resistance mediated by Fra2, exploring its potential as a biomarker and identifying novel molecular targets to overcome KRAS-inhibitor resistance. WP4 will identify a selective Fra2-inhibitor and assess its translational impact.

Expected results

By delving into the role of Fra2 in PDAC, we expect to define how stress adaptivity ultimately contributes to the progression of this recalcitrant disease. We expect to gain insights into molecular mechanisms underlying the interplay between cancer cells and the TME, elucidating how Fra2 fosters PDAC heterogeneity, evolution, and drug resistance. Furthermore, we expect to uncover novel molecular targets suitable for therapeutic intervention.

Impact on cancer

From a translational perspective, completing the proposed studies will directly impact the management of PDAC patients, provide novel biomarkers and broaden our therapeutic arsenal to counteract this deadly malignancy. Additionally, highlighting the stress response as a central feature of PDAC will lay the groundwork for future investigations.

Donatella Romaniello **MFAG - 2023**

Alma Mater Studiorum Università di Bologna

Neutralizing HER3/NRG1 Axis By A Combination Of Therapeutic Aptamers And Monoclonal Antibodies In HNSCC Organoids

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Background

EGFR has been found overexpressed in 80-90% of Head and Neck Squamous Cell carcinomas (HNSCCs) and is associated with poor overall survival and progression-free survival. Surgery and chemo/radiotherapy combined (for HPV-negative HNSCCs) with the anti-EGFR monoclonal antibody cetuximab represent a therapeutic strategy, however, tumors commonly relapse and mechanisms regarding inherent or acquired resistance need to be further elucidated. Immune checkpoint inhibitors like anti-PD1 pembrolizumab and nivolumab have been lately approved by the FDA for recurrent or metastatic HNSCC, but still the role of tumor immunity is not fully understood and currently patients are left with few treatment options.

Hypothesis

Increased HER3 protein level after treatment with cetuximab was observed in HNSCC patients, thus suggesting that this receptor may represent a bypass pathway to the standard of care. Considering that lack of representative models, our hypothesis is to convey our research study into a new viable system to target HER3/NRG1 axis represented by organoids, which can be expanded for long-term remaining genetically and phenotypically stable.

Aims

The aim of this project is to establish a fast and easy way to grow organoids and create a small Biobank of genetically characterized tumors in order to identify new biomarkers, explore mechanisms of drug resistance and apply screening of new drugs for personalized treatment. Particularly, Our goal is to employ PDO to examinate and targeting HER3 axis as a compensatory/bypass signaling pathway responsible for HNSCC resistance to CTX using multiple approaches. Directly neutralizing ERBB3 using an anti-HER3 antibody recently developed by our collaborators. Next, we will explore the use of an innovative nucleic acid-based therapy referred to as aptamers, which we developed upon screening in the last year, and finally with the use of a specific monoclonal antibody targeting the HER3 ligand, NRG1.

Experimental design

In collaboration with Sant'Orsola-Malpighi Hospital we will collect several fresh samples directly from surgery in order to establish and expand few organoids models. All of them will be characterized and HER3, its ligand (NRG1) as well as the other RTKs (receptor tyrosine kinases) expression profile will be defined through several analysis and tested with anti-NRG1 or anti HER3 drugs, including the innovative aptamer, in combination with already approved drugs.

Expected results

Considering the high heterogeneity of HNSCC tumors, the generation of 3D HNSCC organoids will give us an opportunity to have a deeper knowledge of the ERBB signalling and the compensatory signaling pathways.

Particularly, organoids will elucidate the involvement of HER3 receptor and its ligand in the onset of cancer resistance.

Impact on cancer

HNSCC organoids would bypass limitations of the previous old systems and would better resemble morphological and functional HNSCC characteristics. Thus, given the lack of long-term effective treatments, we expect to give a new therapeutic approach and a new hope for HNSCC patients with no treatment option.

Simona Romano

Università degli Studi di Napoli "Federico II"

Insights into the mechanisms of alternative macrophage polarization to circumvent cancer immunotherapy resistance

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Background

TAMs play a pivotal role in promoting cancerogenesis and tumor progression; their targeting has recently emerged as a promising strategy for cancer defeat. Current approaches focus on reducing macrophage infiltration in tumors and reprogramming TAMs from the M2 pro-tumoral to the M1 anti-tumoral phenotypes to kill cancer cells. However, so far, these approaches have failed to improve overall survival. Moreover, given the pivotal role of TAMs in the development of acquired resistance to immunotherapy (IT), there is an urgent need for diagnostic tools to assess these tumor-friendly cells accurately. Unfortunately, in-depth knowledge of TAMs and a signature that can reliably identify them still needs to be improved. We identified a splicing isoform of the FKBP5 gene, FKBP51s, exploited by cancer cells to suppress undesired immunity and highly expressed in the peripheral blood mononuclear cells of tumor patients non-responders to IT. Preliminary experiments of alternative polarization of primary monocytes showed that the FKBP5 gene, constitutively expressed in immune cells, undergoes alternative splicing during the switch from M1 to M2 macrophages.

Hypothesis

The present proposal is based on the central hypothesis that FKBP51s controls the TAMs signaling pathways and supports the immune suppressive tumor microenvironment (TME).

Aims

This proposal points to deciphering the role of FKBP51s in TAM biology with the final aims of 1. develop FKBP51s inhibitors for TAMs reprogramming, and 2. exploit FKBP51s as a univocal biomarker of TAMs and immune tolerance.

Experimental design

As translational activity and metabolic reprogramming are deeply involved in the mechanisms that govern alternative macrophage polarization, the proposal will initially provide mechanistic insights into the role of FKBP5 in these biological aspects. Then, we will test the efficacy of small molecules for FKBP51s targeting. We will employ functional assays and several cell systems to select compounds of interest, including primary PBMCs, a syngeneic mouse melanoma model, and melanoma patient-derived organoids (PDTO). A large study of TAM membranes will also be carried out to identify a signature that can reliably identify TAMs, to be validated on PBMCs and TILs from melanoma patients and PDTO, leading to the definition of TAMs-leading phenotypes involved in IT resistance of melanoma patients.

Expected results

Results will provide elements supporting FKBP51s targeting as a means for reprogramming TAMs. They will drive the production of new drugs with preclinical evidence for their efficacy against melanoma and IT

resistance. Furthermore, results from this proposal will identify TAMs-leading phenotypes to be developed as a diagnostic method for melanoma patient selection and therapy monitoring.

Impact on cancer

The overall project is innovative as it looks toward a precision intervention for melanoma treatment and diagnosis by dealing with a topic of growing impact concerning TAMs. A deep insight-knowledge of TAMs biology and mechanisms making the tumor stronger and invisible to the immune system will have important implications for cancer therapy, introducing a reliable IT biomarker and a promising drug target for partnering with IT.

Eliana Ruggiero

Università Vita-Salute San Raffaele

Harnessing the immunosuppressive leukemic microenvironment to engineer T cells with enhanced anti-tumor functionality

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Background

Despite extensive research on the disease biology, the treatment of acute myeloid leukemia (AML) remains a black hole. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative option, highlighting AML sensitivity to immunotherapy; however, relapse often occur. Chimeric antigen receptor (CAR) T cells have not replicated the success seen in B cell tumors, due to AML heterogeneity, absence of ideal surface antigens and poor lymphocyte persistence.

Dressing up T cells with tumor-specific T cell receptors (TCRs) could overcome some of these roadblocks, by enabling sensitive antigen recognition and enhanced cell persistence. However, the profoundly altered bone marrow microenvironment in AML further challenges immune therapy efficacy.

Hypothesis

Given the crucial role of donor-derived T cells in driving anti-tumor responses in HSCT, we hypothesize that the impressive variety of mechanisms employed by tumor cells to evade immune recognition could inform the design of "smarter" T cell therapies. We will tackle three major Achille's heels hindering the success of current immunotherapies: impaired CD4+ T cell functionality, downregulation of human leukocyte antigens (HLA) on cancer cells, and deregulated release of immunosuppressive molecules in the bone marrow. By addressing these issues, we will develop an advanced T cell product (TCR T cells v2.0).

Aims

Our goal is to generate novel living drugs represented by engineered CD4+ and CD8+ T lymphocytes that sense immunosuppressive cues in the leukemic milieu and react by putting in place more powerful anti-tumor cytotoxic weapons.

Experimental design

We will integrate TCR repertoire sequencing, single cell transcriptional and epigenetic profiling, 3D cultures, high throughput phenotypic T cell analysis, genome editing, synthetic biology, and murine AML models. Through this multidisciplinary approach we will identify anti-tumor HLA class II-restricted TCRs and design therapeutic T cell circuits that sense immunosuppressive signals (transforming growth factor β, TGFβ and Adenosine) and enhance anti-tumor T cell functions (expression of an HLA class II-restricted tumor-specific TCR and induction of HLA expression on leukemic blasts). Our approach is geared at turning the leukemic cells from an enemy to an ally for T lymphocytes.

Expected results

Our study will guide the development of more effective therapies for the treatment of AML patients by: 1) building a TCR library that encompasses diverse antigens and HLA class II restrictions to unleash the anti-tumor potential of CD4+ T lymphocytes, key players for leukemia immunosurveillance; 2) developing new

cellular products that leverage tumor immune suppression to reinforce anti-tumor T cell functionality; 3) identifying novel pathways/mechanisms involved in cancer/T cell evolution and immune evasion.

Impact on cancer

This project holds significant translational potential: 1) Newly discovered TCRs will be validated for clinical use and made accessible to European clinical centers to combat tumors. 2) Inducible restoration of HLA expression, combined with the targeting of multiple HLA I and II tumor antigens and with the disruption of disease-relevant inhibitory receptors (IRs), may overcome AML heterogeneity and counteract tumor escape mechanisms. 3) Since TGFβ, Adenosine, IRs, and HLA downregulation suppress immune responses in various cancers, our findings could represent a springboard for developing innovative, long-lasting therapies for additional tumors.

Jolanda Sarno **Start-Up - 2022**

Fondazione M. Tettamanti M. De Marchi Onlus

Developmental heterogeneity as a key determinant of treatment resistant cells in childhood acute lymphoblastic leukemia

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Background

Despite the exciting milestones achieved in the last decades for the treatment of acute lymphoblastic leukemia (ALL), relapsed ALL still remains one of the leading causes of cancer-related death in children. To further improve childhood ALL outcomes, it is critical to identify and understand cellular populations causing treatment failure. Minimal residual disease (MRD) detection after remission induction therapy negatively affects outcome and its measurement is used as predictor of relapse in risk-assignment. MRD cells are rare thus requiring a single cell approach to find and query them. Current clinical MRD approaches do not inform why these leukemia cells are resistant. I herein propose a longitudinal MRD sample analysis at single-cell resolution of a large cohort of patients to identify which are the resistant cells and which is the best way to effectively target them.

Hypothesis

Based on previous findings and preliminary data, I hypothesize that developmental heterogeneity is crucial in treatment failure. Diagnostic pre-B ALL cells relying on active pre-BCR signaling are treatment resistant and represent MRD cells. These cells might persist during treatment due to their metabolic flexibility. Moreover, other sub-clones might also accumulate genetic alterations that lead them to emerge under the pressure of the treatment and be therefore responsible of the relapse.

Aims

To prove my hypotheses, I aim to first identify relapse associated cells at early MRD timepoints and characterize their features. Second, I will investigate mechanisms of resistance to glucocorticoids, drugs heavily used during the remission induction therapy, by focusing on metabolic rewiring of resistant cells. Finally, I aim to determine the clonal identity and gene signature of relapse-associated cells.

Experimental design

I have collected an unprecedented cohort of 50 B-ALL patient samples, at different timepoints of treatment: diagnosis, MRD (day 8 and day 15) and relapse. These samples will be profiled using multi-omics approach. By single cell proteomic analysis, I will identify relapse associated cells to determine if they are already present at the time of diagnosis and persist due to treatment resistance or if they emerge during therapy. On a subset of these samples (n=10), I will study treatment induced metabolic flexibility at single cell level to uncover metabolic heterogeneity. Finally, I will integrate all this information with mutational identity and transcriptome analysis of resistant cells.

Expected results

The comprehensive understanding of phenotype, signaling, cellular metabolism, mutations, and gene signature of relapse-associated cells in clinically annotated longitudinal samples, will unravel novel and key

insights on how resistant cells are selected and/or adapted during therapy.

Impact on cancer

On a broader level, the integration of a qualitative evaluation of MRD with the quantitative detection performed with standard methods will further improve the risk stratification of the patients and inform about mechanisms associated with treatment failure. Identifying which cells to target at early stages of resistance will unlock actionable therapeutic options, ultimately preventing relapse and improving the outcome of children with B-ALL.

Angela Maria Savino **Start-Up - 2023**

Università degli Studi di Milano - Bicocca

Targeting the Dysregulated Metabolic Program in Acute Leukemias

Background

The recent discovery of oncogenic mutations in metabolic genes, and the development of accurate methods for metabolic profiling, have prompted a renewed interest in the rational design of dietary-based therapies.Acute Myeloid Leukemia (AML) represents the most frequent cause of leukemia-related deaths in adults,especially upon relapse in the bone marrow and extramedullary sites. IDH1 and IDH2 are mutated in up to 20% of AML patients. Despite FDA-approved IDH1/2-specific inhibitors, less than 40% of mutant IDH AML patients respond. The metabolic effects of IDH1/2 mutations are complex and understudied. The evolution of clinically overt B-cell precursor acute lymphoblastic leukemia (BCP-ALL) includes initiating pre-leukemic genetic events followed by a poorly understood transition to leukemia, which can reoccur in children after failing to completely eradicate the pre-leukemic clones.

Hypothesis

Fructose aberrantly accumulates in the leukemic bone marrow compared to healthy subjects. I hypothesize that sugar and lipid metabolism can be reprogrammed in leukemic patients upon fructose exposure, unravelling specific metabolic vulnerabilities, inaccessible if other sugar sources are available.

Aims

This project aims to investigate the impact of fructose metabolism on the subversion of physiological hematopoiesis in the context of leukemic initiation (BCP-ALL), progression and metastasis (AML).

Experimental design

This proposal will be structured in four working packages (WP1-4). The first three will address disease relapse and metastasis in mutant IDH1-2 AML patients.Developing in vitro and in vivo disease models and performing molecular phenotyping using CyTOF, RNAseq, and MALDI-imaging, I will investigate sugar and lipid metabolic vulnerabilities in the leukemic bone marrow exposed to fructose (WP1); profile fructose-dependent gene regulatory networks (WP2); and spatially resolve the metabolic determinants of extramedullary metastatic niches in the context of a fructose-based treatment (WP3).A fourth working package (WP4) will investigate the role of fructose metabolism in the transition from pre-leukemia to overt BCP-ALL, via functional genetics and molecular epidemiology approaches.

Expected results

I expect to demonstrate effective and safe remission in pre-clinical models of mutant IDH AML1-2 through co-targeting of fructose-induced serine and lipid synthesis vulnerabilities, as well as to determine the association between sugar intake, mutational status, and disease outcome in clinical cohorts for predicting patient eligibility for such metabolic treatments (WP1);to identify master regulators of fructose-dependent gene regulatory networks in a mutant IDH1-2 leukemic background (WP2); to nominate and test extramedullary niche-specific metabolic vulnerabilities in AML models with mutant IDH1-2 (WP3); to prove

that fructose metabolism primes pre-leukemic B cells to leukemic transformation in patient-derived HSC ex-vivo (WP4).

Impact on cancer

This project can lead to personalized therapies based on patient-specific metabolic requirements and leukemia dependencies to overcome mutant IDH1-2 AML relapse and metastasis in adults (WP1-3) and eradicate pre-leukemic clones causing BCP-ALL recurrence in children (WP4). We will pursue dietary interventions (or interference) complementing current chemotherapy strategies with the potential to increase efficacy and minimize toxicity. Preliminary experiments indicate that insights from this project may have broader applicability for the cancer field beyond leukemias.

Marta Serafini **Start-Up - 2024**

Università degli Studi di Torino

Selective hypoxia-triggered intratumoural formation of chemotherapeutic agents in colorectal cancer

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Background

Systemic exposure to small molecule anticancer drugs yields severe and dose-limiting toxicities. It is therefore imperative to find strategies to increase the concentrations that reach the tumour while decreasing those reached in other organs. The present proposal aims at taking this to the extreme: directly forming the drugs inside the tumour using the tetrazine-dienophile click ligation. This reaction is fast and compatible with living systems. Yet, for this strategy to succeed, it is crucial to have spatial control over the reaction.

Hypothesis

The working hypothesis is that the tetrazine-dienophile ligation can be spatially controlled by caging the reactants of this click reaction with chemical groups that are unstable in the tumour microenvironment. Specifically, I will initially concentrate on triggering the reaction with hypoxia and will focus on colorectal cancer, despite hypoxia characterizes most other solid tumours.

Aims

The specific aim is to use a hypoxia-triggered tetrazine-dienophile ligation (i) to release two distinct cytotoxic drugs, irinotecan and oxaliplatin, solely inside hypoxic colorectal cancers; and (ii) to form directly inside hypoxic cells active protein degraders for ATR, a crucial effector of the DNA damage response pathway. As the latter is involved in patient refractoriness to irinotecan, eventually the two strategies will be combined in a unique approach, in which a single click reaction induces the release of irinotecan and the formation of an ATR protein degrader.

Experimental design

The starting points of the project will be (1) the hypoxia-activated groups I characterized during my post-doc in Oxford; and (2) the preliminary data I collected in the past months in Turin. The project will employ a reiterative approach of in silico studies, chemical synthesis and biological screening for the refinement of candidate molecules. Selected compounds will be moved to patient-derived colorectal cancer organoids to evaluate the efficacy in a model which recapitulates the complexity and heterogeneity of cancer, before testing them in patient-derived xenografts.

Expected results

While improvement of the benefit/risk ratio of irinotecan, oxaliplatin and the discovery of ATR degraders will be the specific focus, the chemical biology approach proposed has the ambition to provide answers to other questions: (1) Are organoids able to recapitulate the hypoxic environment of tumours? (2) Are there specific mechanisms of resistance which are peculiar to hypoxia? (3) Is the removal via proteasomal degradation of ATR effective enough to re-sensitize tumours which are refractory to chemotherapy? (4) Can other

peculiarities of the tumour be used to orchestrate a chemical reaction?

Impact on cancer

The development of a controllable version of the tetrazine-dienophile ligation would represent a versatile and generalizable platform that would allow drug targeting in hypoxic tumours. Furthermore, by expanding the click ligation to other triggers which are peculiar of the tumour microenvironment, the project would generate a toolkit of ligations that address the heterogenicity of cancer. Overall, this strategy would allow to improve the efficacy and safety of old and new drugs, as well as use molecules which at present are thought to be too toxic to be administered systemically.

Alessandro Sessa **MFAG - 2021**

Università Vita-Salute San Raffaele

Targeted epigenetic strategy for the treatment of glioblastoma multiforme

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Background

Glioblastoma multiforme (GBM) is the most common and lethal brain cancer in adults (1-5 cases per 100,000 people per year, 12-15 months of median survival). This is due, in addition to the aggressiveness of the disease, to the (in)efficacy of current therapies that increase the overall survival only marginally. Patients usually undergo tumor resection followed by adjuvant radio- and chemo-therapies, that can't prevent recurrences. It has been proposed that some stem cell-like cancer cells with tumor-initiating potential remain in preserved tissue, resist the adjuvant treatments, leading to the re-appearance of the disease.

Hypothesis

I generated a synthetic transcription factor based on SOX2 protein that, functioning as an epigenetic repressor (SOX2 Epigenetic Silencer, SES), should inhibit a network of genes important for tumor maintenance and growth, including the molecular targets of SOX2 itself within cancer stem cells. I hypothesize that a treatment based on SES administration in vivo, e.g. post-surgery brain, can extinguish the GBM resistant cells and minimize the risk of tumor reappearance, without risk for the surrounding (healthy) cells.

Aims

I propose to validate my hypothesis, at the pre-clinical level, with the following work packages:
WP1: To define the functional role of SES in coordinating the anti-tumor effect.
WP2: To prove the SES anti-tumor activity in human settings and in vivo models.
WP3: To assess and increase the safety of the treatment.

Experimental design

We will validate our strategy to be detrimental for GBM using in vitro and in vivo models. Among the first, we will adopt 3D GBM-cortex organoids while among the latter we will exploit human GBM xenograft in mice as well as a syngeneic GBM mouse model. We will also investigate the molecular consequences of SES within the chromatin and its impact on tumor heterogeneity with a single cell level analysis. Finally, we will explore possible undesired SES effects on healthy brain cells and the possibilities of vector amelioration to make the strategy safer.

Expected results

We expect to demonstrated SES validity in limit tumor growth, expansion, and recurrence in compelling models of GBM. We will also indicate which subpopulations within experimental GBMs are either particularly resistant or sensitive to the treatment. We expect to map those changes in epigenetic traits and transcriptional functional for SES effect. We will also provide elements to both evaluate and increase the safety in the SES usage, particularly regarding normal cell survival, functioning, and brain activity.

Impact on cancer

Current therapies against aggressive high-grade gliomas fail due to fatal tumor recurrence. Here I propose to properly assess the efficacy, the molecular functioning, and the safety of a gene therapy-like strategy to eliminate the cancer stem cells, the exact responsible for tumor return. Moreover, I want also to refine the knowledge on resistance to therapies. Indeed, the reaction of the GBM cells to the molecular makeover induced by SES may give hints on their adaptive capabilities. I based my strategy on rationale stemming in brain development, cancer stem cell biology, epigenetics, and biotechnological engineering, feeling that it may represent a game-changer in the field of brain cancer.

Valentina Silvestri **MFAG - 2024**

Università degli Studi di Roma "La Sapienza"

Molecular characterization of BRCA2-associated cancers: role of environmental and sex-related determinants

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Background

BRCA1 and BRCA2 genes have been initially identified as hereditary breast and ovarian cancer risk genes, and traditionally considered as a single entity. However, differences between the two genes in conferred risks, cancer spectrum, molecular characteristics, and response to therapy have been starting to emerge. The more heterogeneous spectrum of BRCA2-associated cancers, including cancers arising in non-reproductive tissues, is still largely unexplored at genetic and molecular level, deserving specific investigation to implement a more appropriate, personalized and inclusive clinical management for BRCA2 pathogenic variant (PV) carriers.

Hypothesis

Recent studies and preliminary data support the hypotheses that:

- BRCA2-associated cancer risk in non-reproductive tissues may be due to gene-environment interactions;
- BRCA2-associated mutational, structural and microenvironmental profiles may be different from those in BRCA1-associated cancers, providing possible gene-specific actionable targets;
- sex-related determinants may be key factors in BRCA2-associated cancers, showing a possible bias towards the male sex.

Aims

'- To evaluate gene-environment interactions on cancer risk in BRCA2 PV carriers
- To characterize BRCA2-specific, possibly actionable, tumor molecular features
- To identify sex-specific determinants of BRCA2-associated cancers

Experimental design

Pancreatic cancer, arising in a non-reproductive tissue and associated with BRCA2 PVs in both sexes, will be the initial model for studying the impact of environmental, molecular and sex-related determinants associated with BRCA2. Gene-environment interactions will be investigated using data from the UK biobank. Genomic, transcriptomic and epigenetic profiles will be investigated by short-read and long-read sequencing. Tumor and stromal microenvironment will be investigated using spatial transcriptomics. Pan-cancer cohorts will be used to validate results.

Expected results

This project is expected to provide fundamental and translational knowledge about BRCA2-associated cancers as a distinct entity, different from the BRCA1 counterpart, and on relevant yet understudied issues in cancer research, such as the impact of gene-environment interactions and sex-related determinants. Using pancreatic cancer as a study model will help in the discovery of molecular features less biased by reproductive-related factors. By addressing important knowledge gaps with advanced omics technologies,

results from this project have the potential to inform the development of more personalized and effective clinical strategies.

Impact on cancer

Implementing a molecular-based classification of cancers represents a shift of paradigm in the field, with a key impact, in terms of clinical outcomes, for the heterogeneous group of BRCA2-associated cancer patients. A deeper understanding of BRCA2-environment interactions will impact on improving precise and individualized preventative strategies for BRCA2 PV carriers. The identification of somatic molecular alterations, distinguishing BRCA2 from BRCA1 deficiency, could inform future clinical research targeting gene-specific vulnerabilities, advancing precision treatment. Considering the effect of sex-related factors will help in the much-needed establishment of a gender-oriented clinical management.

Giorgia Simonetti **MFAG - 2023**

Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori", IRST S.r.l. I.R.C.C.S.

Exploring polyamine metabolism as selective vulnerability for therapeutic combinations in acute myeloid leukemia

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Background

Acute myeloid leukemia (AML) cells undergo metabolic reprogramming and are susceptible to metabolic changes, as induced by Venetoclax (Ven) and Azacitidine (Aza) combination, the current standard of care for elderly/unfit patients that, however, does not cure them. Among AML metabolic hallmarks, we recently uncovered alterations in polyamine metabolism that also shapes tumor-mediated immune response, while being conditioned by the microbiota.

Hypothesis

Recent findings and our preliminary data suggest potential selective vulnerabilities to inhibition of polyamine metabolism across AML molecular subtypes and in combination with Ven/Aza. The definition specific leukemia metabolic dependencies may definitively overcome on-target off-tumor toxicities that currently hamper the clinical applicability of therapeutics in the field of cancer cell metabolism and open a therapeutic window for combined Ven/Aza/polyamine inhibition in AML. Moreover, we hypothesize that a reinforced anti-leukemia T cell response and the microbiota composition may contribute to treatment efficacy when inhibiting polyamine metabolism (also in combination with Ven/Aza). Finally, we believe that preclinical models accounting for the complexity of reactions occurring through tumor-host interactions may relieve the repetitive inconsistency between preclinical and clinical results on metabolism-targeting agents.

Aims

The project aims to explore polyamine metabolism as selective vulnerability for therapeutic combinations in AML. Specifically, it aims to: (i) generate 3D bone marrow models suitable to address the metabolic perturbations occurring in the leukemia microenvironment and reliably evaluate the efficacy of therapeutic combinations; (ii) study the pathogenic role of dysregulated polyamine metabolism in AML, both as leukemic fuel and immunosuppressive mechanism; (iii) exploit polyamine targeting to improve Ven/Aza combination efficacy, by unraveling specific dependencies, towards metabolic-oriented personalized AML therapies.

Experimental design

To accomplish the research aims, the project is developed along three integrated work-packages (WPs). In WP1 we will structure and characterize autologous multicellular 3D bone marrow models obtained by combining cell bioprinting and cultures. In WP2 we will inhibit polyamine metabolism by pharmacological approaches and cell engineering and study its functional consequences on leukemic cells and the immune response both ex vivo, in the 3D model and in vivo, in immunocompetent mice. In WP3, we will study the combination of Ven/Aza and polyamine metabolism inhibition ex vivo and in vivo, and we will predict biomarkers of response to the triplet, taking into account the disease molecular feature and the microbiota contribution to the polyamine reservoir.

Expected results

The project will uncover the functional role of polyamine metabolism in AML. By evaluating drugs already known to be suitable for clinical combinations, capturing selective vulnerabilities of AML molecular subgroups and defining predictive markers of ex vivo response, the results will enable a direct translation of our metabolic-oriented personalized approach into clinical trials of Ven/Aza/polyamine metabolism inhibitors. Moreover, we will provide a preclinical model ameliorating personalized drug testing and accelerating drug development or repurposing.

Impact on cancer

The project answers the urgent medical need of early and personalized interventions able to induce deep and durable clinical responses, while sparing toxicities in elderly/unfit AML patients that currently have a 5-year survival of 5-10% and no therapeutic options at disease relapse/refractoriness.

Andrew Smith

Università degli Studi di Milano - Bicocca

Dissecting the tumour-immune landscape of ccRCC: the role of altered lipid metabolism in resistance to immunotherapy

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Background

Renal cell carcinoma (RCC) is among the top ten most commonly diagnosed cancers worldwide and clear cell (ccRCC) is the predominant histological subtype, representing 75% of all cases and the majority of cancer-associated deaths due to the high rate of disease recurrence and resistance to conventional cytotoxic chemotherapy and radiotherapy. Whilst this landscape has improved somewhat due to the emergence of immune checkpoint inhibitors (ICIs), a substantial proportion still do not respond to these therapies and patients who initially do respond eventually progress, meaning that alternative strategies may be required in order to improve clinical outcomes.

Hypothesis

The tumour immune microenvironment has become a key research area in ccRCC due to its important role in immune surveillance and it is becoming increasingly evident that the presence of certain subsets of T cells and TAM phenotypes may be associated with poor outcome and treatment resistance in ccRCC. However, most studies have only focused on the presence of immune cells, which are surrounded by a heterogeneous and complex tumour background. It is our belief that the presence of certain subsets of immune cells communicate with phenotypically distinct tumour cells in close proximity, with the latter displaying aberrant metabolism of lysophosphocholine and phosphatidylserine species, and this, collectively contributes to innate tumour immunity in ccRCC.

Aims

The principal objectives of this project are to:

1) Comprehensively characterise the single-cell lipid cell phenotypes present within the complex tumour-immune landscape of ccRCC and determine which are associated with response to immunotherapy

2) Describe the dysregulated lipid metabolism pathways and cell-to-cell molecular interactions which drive resistance to immunotherapy

Experimental design

To reach these objectives, the project will be structured into the following work packages (WPs):

1) Mapping the tumour-immune landscape of ccRCC with advanced spatial multi-omics

WP 2) Pinpointing tumour cell phenotypes and immune cells which correlate with resistance to immunotherapy

WP 3) In-depth lipidomic characterisation of the tumour and immune cell phenotypes which are associated with response to immunotherapy in ccRCC

WP 4) Modelling immune-tumour cell interactions and immune modulation using an in vitro heterotypic 3D cellular system of ccRCC

WP 5) Enlighten the molecular mechanisms that drive resistance to immunotherapy in ccRCC using an

integrated systems biology approach based on the multi-omics datasets

Expected results

This integrated study will improve our understanding of the molecular communications which occur between phenotypically distinct tumour and immune cells within the highly complex and heterogeneous context of ccRCC and unravel the lipid metabolism pathways which drive the immunogenic environment and resistance to immunotherapy.

Impact on cancer

The proposed study will elucidate more specific signalling pathways, initiated by dysregulated lipid metabolism, that may be targeted in order to provide alternative treatment options in cases which are currently associated with a bleak clinical outlook. Moreover, it is envisioned that this dysregulated lipid metabolism is also implicated in the regulation of known immune checkpoints and their downstream effects and could be exploited in order to improve the efficacy of these therapeutic strategies.

Federica Sodano **SIS - 2023**

Università degli Studi di Napoli "Federico II"

targeting arginaSe 2 tO disrUpT tHe imMunosUppreSsive Tumour microEnvironMEnt and pRomote Gastric cancEr response

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Background

Gastric cancer (GC) is the fifth most common malignancy and the third leading cause of cancer death worldwide. Immunotherapy based on immune checkpoint inhibitors, such as PD-1 and PD-L1, has undoubtedly revolutionized the treatment of some previously incurable cancers and has become one of the mainstays of innovation in cancer therapy. However, only a small percentage of patients can benefit from immunotherapy because of the establishment of resistance mechanisms as those related to the immunosuppressive tumor microenvironment (TME). It has been recently reported that melanoma-associated fibroblasts impair CD8+ T cell function and modify expression of immune checkpoint regulators via increased arginase (ARG) activity. Moreover, ARG isoform 2 (ARG2) controls regulatory T (Treg) cell metabolic fitness and correlates with their immunosuppressive function in cancer.

Hypothesis

ARG2 is therefore a valuable target for T-cell-based cancer immunotherapies. Because ARG1 and ARG2 are structurally similar, the development of an ARG2-specific inhibitor has not borne fruit. Taking advantage of the different subcellular localization of the two isoforms, specific inhibition of ARG2 could be developed by synthesizing molecular hybrids capable of delivering the most promising ARG inhibitors (ARGi) into mitochondria.

Aims

This project proposal aims to both identify new and more potent inhibitors (nARGi) and molecular hybrids targeting ARG2 to improve the success rate of immunotherapies and contribute to the development of patient-centred personalized therapy.

Experimental design

The design of new and more potent inhibitors (nARGi) will be carried out using computer-assisted drug design techniques. The identified scaffolds (nARGi) will subsequently be targeted to mitochondria (mt-nARGi) by covalent bonding with high trophism vectors for mitochondria. As an innovative strategy, the effect of ARG inhibitors on the native TME and GC-specific immune response in GC-Patient Derived Organoids will be evaluated.

Expected results

The expected results from evaluating the effect of ARG inhibitors on TME composition and activation and GC-specific immune response will help elucidate the onset, development, and metastasis of GC. In addition, for the first time, organoids derived from GC patients (GC-PDOs) will be used as a model.

Impact on cancer

The identification of new molecular hybrids targeting ARG2 would aid disease management, improve the

success rate of immunotherapies, and contribute to the development of target therapies, with benefits in quality and life expectancy for patients with GC and beyond.

Maura Sonego **MFAG - 2020**

Centro di Riferimento Oncologico - Aviano - I.R.C.C.S.

USP1 and DNA damage repair: exploring new interactions in ovarian cancer progression and therapy response.

Background

Epithelial ovarian cancer (EOC) is the most lethal gynaecologic malignancy for its late diagnosis and the occurrence of chemoresistance. Despite some important clinical advances, the survival of patients with chemoresistant EOC has not been significantly improved in the last 30 years, suggesting that alternative approaches are urgently needed.

We recently demonstrated that, upon platinum (PT) treatment, the de-ubiquitnase USP1 is phosphorylated on its S/TQ motifs. This phosphorylation mediates most of USP1 effects in protecting EOC from PT-induced cell death and in favouring their metastatization. Here, using a proteomic approach, we have identified several proteins interacting with USP1 in a S/TQ phosphorylation-dependent manner following PT-treatment. Among them, we will focus on two key players of the DNA damage response (DDR) (i.e. BARD1 and Mre11).

Hypothesis

We reasoned that USP1 is a central protein in the response to PT in EOC and that its activity is necessary for cell survival following PT-treatment. Our hypothesis is that, following PT-treatment, USP1 expression regulates the different steps of the DDR interacting with and regulating the expression and function of BARD1 and Mre11.

Aims

Our main goals are to dissect the molecular mechanisms by which USP1 is regulated under PT pressure and participates in the response to PT regulating DDR. With the clarification of these mechanisms we will improve the knowledge on PT-resistance and possibly impact on the management of EOC patients.

Experimental design

Based on literature and solid preliminary data, we divided the project in 3 interconnected but independent WPs to minimize the risks of failure. WP1 will clarify how the expression of USP1 is regulated in EOC and which is the role of the BET protein BRD4 in its transcriptional regulation. Then we will dissect the molecular mechanisms by which USP1 is phosphorylated on S/TQ sites, likely by ATM/ATR kinases. Finally, we will explore the ubiquitinome to better clarify the role of S/TQ phosphorylation for USP1 activity. In WP2 we will dissect the interaction between USP1 and BARD1 or Mre11 trying to unveil the significance of this interaction in the context of DDR. To this aim we will use several in vitro approaches and models including available USP1 knock-out and knock-in EOC cells in which the expression of BARD1 or Mre11 will be modified. WP3 will be dedicated to dissect the significance and the clinical relevance of the in vitro studies using a large collection of available primary tumors samples and the most appropriate syngeneic (ID8 modified cells) or xenograft EOC mouse models.

Expected results

We expect to validate a role USP1 in the coordination of PT-response in EOC elucidating its relationship in new processes of DDR. Being USP1 targetable with specific small molecules we expect to provide new evidences for possible therapeutic approaches in PT-resistant EOC.

Impact on cancer

With this project we will direct impact on a critical clinical unmet need that is the acquired PT resistance in EOC. If successful our work could result in the identification of new possible markers (such as DNA damage repair markers) and/or therapeutic targets that might positively impact on the management of EOC patients.

Paola Storti **MFAG - 2020**

Università degli Studi di Parma

Unraveling the mechanism behind the progression of indolent monoclonal gammopathies to multiple myeloma.

Background

Multiple myeloma(MM) is the malignant stage of plasma cell(PC) dyscrasias that is preceded by monoclonal gammopathy of undetermined significance(MGUS) and smoldering MM(SMM). MGUS and SMM are biologically heterogeneous including patients with low rate of progression to active MM, as well as patients that progress within five years from diagnosis. Up to date, the studies of PCs genomic and transcriptional profiles lack to identify a feature that can reliably distinguish MGUS/SMM patients who will progress to MM

Hypothesis

Alterations of bone microenvironment leading to bone destruction are the hallmark of MM as compared to MGUS/SMM. Thus we hypothesize that transcriptional and functional modifications of osteoblasts(OB)s and mesenchymal stromal cells(MSCs) in longitudinal studies from MGUS/SMM stage to MM are an important topic to define the bone microenvironment role in the progression and to identify new markers of high risk progression to active MM.

Aims

The aim is to identify the biological mechanism related to the microenvironment involved in the progression of MGUS/SMM to active MM with a possible translational and therapeutic impact in order to identify those patients with higher risk for progression that could benefit from an early treatment. Finally, our project will permit to study the impact of the microenvironment in the progression to MM in preclinical models.

Experimental design

1)We will perform a transcriptional analysis by single cell RNAseq on paired primary non-hemopoietic cells and mutational analysis on PCs of a cohort of progressed and not-progressed MGUS/SMM in order to identify possible alterations in the BM microenvironment.

2)The genes, highlighted from point 1, will be modulated in MSC, OB cultured both alone or with PCs. A battery of cancer-relevant assays will be performed to understand the mechanisms by which the candidate alterations contribute to the progression from MGUS/SMM to MM.

3)MSCs transduced for the selected alteration and PCs will be implanted in different xenograft MM models using both cell lines and primary human cells. Tumor growth and PCs clonal distributions assessed.

4)In a cohort of high-risk MGUS/SMM patients, primary MSCs will be phenotypically characterized as immune-check point molecules, and MM-relevant molecules. The results will be stratified and analyzed based on the progression or not in the follow-up period.

Expected results

The project will lead to the identification of transcriptional and functional specific bone microenvironment features that promote the progression to active MM. The study will contribute to understand the principal

steps of tumor progression from asymptomatic forms to active MM. Finally, the project will lead to the identification of possible biomarker of progression to active MM.

Impact on cancer

The application of our approaches (bioinformatics, in vitro and in vivo) will result in the identification of mechanisms of the progression to MM driven by the bone microenvironment, of new molecular markers that can lead to new diagnostic, prognostic and therapeutic strategies, thus with an immediate clinical impact in the management of high risk MGUS/SMM patients. These new factors could identify a subset of MGUS/SMM patients that have an increased risk of progression from diagnosis.

Marco Tagliamento **MFAG - 2024**

Ospedale Policlinico San Martino - IRCCS per l'Oncologia

Unveiling clinical and mechanistic implications of mutations in clonal hematopoiesis genes in patients with solid tumors

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Background

Clonal hematopoiesis (CH), characterized by somatic mutations in hematopoietic stem cells leading to clonal expansion, is primarily studied in hematologic disorders but is gaining attention in solid tumors. Emerging data suggest that CH may influence the tumor microenvironment (TME) and patient responses to anticancer therapies, particularly immune checkpoint inhibitors. Understanding the interplay between CH and TME could provide insights into treatment outcomes in solid tumors like lung and breast cancer.

Hypothesis

We hypothesize that CH mutations in patients with solid tumors actively modify the TME and host characteristics, impacting clinical outcomes and the efficacy of immunomodulatory therapies.

Aims

1) Retrospective Analysis: Assess CH mutations in paired blood and tissue samples to evaluate their distribution and correlation with clinical-pathological characteristics and treatment outcomes.
2) Prospective Study: Confirm retrospective findings in treatment-naïve patients to exclude confounding effects of prior therapies.
3) Efficacy and Toxicity Analysis: Investigate the relationship between CH presence in tissue/blood and the differential efficacy and toxicity of various anticancer regimens.
4) Mechanistic Studies: Explore the functional implications of key CH mutations on the TME at cellular and molecular levels.

Experimental design

This study combines retrospective and prospective analyses. In the retrospective phase, data from the ROME trial (NCT04591431) will be used to examine CH mutations in blood and tissue, assessing their impact on treatment outcomes and transcriptomic profiles. The prospective phase involves genomic profiling of treatment-naïve patients with lung and breast cancer, analyzing CH mutations in tissue and blood. Mechanistic studies will include in vitro and in vivo models to investigate the role of CH in modifying the TME and its components, including tumor-infiltrating lymphocytes and gene expression signatures. The project will leverage NGS, cytofluorimetry, and CRISPR/Cas9 technology.

Expected results

We expect to demonstrate that CH mutations correlate with distinct clinical outcomes in patients with solid tumors. Retrospective analyses will reveal the distribution of CH mutations and their associations with clinical and molecular characteristics. Prospective studies will confirm these findings, potentially identifying CH as a biomarker for treatment efficacy and toxicity. Mechanistic studies will elucidate the role of CH in modulating the TME, providing insights into how these mutations influence immune responses and tumor

behavior.

Impact on cancer

This research could significantly impact cancer treatment by identifying CH as a potential biomarker for patient stratification and therapy selection. Understanding the influence of CH on the TME may lead to novel therapeutic strategies, enhancing the efficacy of current treatments, especially immune checkpoint inhibitors. By integrating CH into clinical practice, we aim to improve treatment outcomes in hard-to-treat solid tumors, contributing to personalized cancer therapy.

Simone Tamburri **MFAG - 2021**

Istituto Europeo di Oncologia I.R.C.C.S. S.r.l.

Characterization of the oncogenic H2A ubiquitination and its underlying regulatory mechanisms

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Background

Genetic mutations targeting epigenetic mechanisms that modify and remodel the chromatin environment at regulatory elements are hallmarks of cancer, with epigenetic regulators representing one of the most frequently mutated families.

The Polycomb machinery is the major repressive mechanism active in facultative heterochromatin. Central to this, is the mono-ubiquitination of histone H2A at Lysine 119 (H2Aub1), deposited by different forms of the Polycomb Repressive Complex 1 (PRC1) and removed by the PR-DUB complex. H2Aub1 levels are kept in check by these opposing activities with high biochemical heterogeneity through mechanisms that are poorly understood. Both activities are essential for development but do not fully display simple antagonistic phenotypes.

Hypothesis

We found that BAP1 mutated tumors and SS18-SSX driven SS represent two distinct oncogenic conditions in which H2Aub1plays a crucial role. In both cases the etiological mutations result in profound reshaping of the epigenome affecting cell transcriptional identity. This places H2Aub1 deposition and its homeostatic regulation in a central position in sustaining cancer development. Since general inhibition of PRC1 activity affects general cell viability, targeting specific forms (sub-complexes) of PRC1 could become an attractive strategy. However, we lack knowledge about the role played by the high degree of biochemical complexity that govers these activities and the underling molecular details involved in sustaining cancer development. Despite the composition of these ensembles have been characterized, very little is known about the biochemical features that govern their assembly, sustain their targeting to chromatin and enzymatic activity under physiological and pathological conditions. This information is essential to comprehend their activity and become crucial for designing new therapeutic strategies.

Aims

This project aims to uncover the mechanisms that connect PcG repression to deposition of H2Aub1 in two distinct oncogenic conditions in which H2Aub1 homeostasis is disrupted: i) the frequent mutations of the tumor suppressor BAP1 - the catalytic activity of PR-DUB - reported in several cancer types; and ii) in presence of an aberrant reading of H2Aub1 modified nucleosomes by the expression of the SS18-SSX oncogenic fusion protein that drives Synovial Sarcoma (SS) development.

Experimental design

The project will define the critical role played by PRC1 in sustaining both BAP1 mutated and SS cancer development. The project will characterize the biochemical properties of PR-DUB and PRC1 macromolecular ensembles, determining their topological assembly. We will use these biochemical information to uncover at genome-wide level the mechanisms that connect PRC1 activity with oncogenic H2Aub1 upon BAP1 loss

and SS18-SSX fusion expression to uncover mechanisms of activity and associated molecular vulnerabilities.

Expected results

This project will provide deep molecular understanding about a central activity that sustain the oncogenic mechanisms driving the development of both BAP1-mutated and SS18-SSX tumors and their link with PRC1 activity. This information will uncover new molecular vulnerabilities and set the basis for the potential design of new therapeutic interventional projects.

Impact on cancer

The project will provide unprecedented molecular understanding of the role played by PRC1 in regulating the oncogenic activities in the context of BAP1-null tumors and Synovial Sarcomas, uncovering structural and functional vulnerabilities that could be used to design new strategies for cancer therapy.

Marcella Tazzari **MFAG - 2021**

Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori", IRST S.r.l. I.R.C.C.S.

Unveiling the function of tertiary lymphoid structures and associated CXCL13 to enhance immunotherapy in solid tumors

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Background

Cancer Immunotherapy (CI), specially tackling the tumor microenvironment (TME), has improved the treatment of multiple types of solid tumors. However, only a minority of patients achieve long-term benefit. By definition, an immune-rich/inflamed TME will expect to parallel a higher degree of response to CI, but this does not reflect the clinical scenario. Of note, inflamed tumors often express ectopic tertiary lymphoid structure (TLS). The heterogeneity and functional complexity of these immunologic "hub" and the intratumoral role of CXCL13, one of the major TLS-associated chemokines, is far from being defined, making their role in tumor still questionable.

Hypothesis

We hypothesize that intra-tumoral inflammation can result in different functional types of TLS with variable impact on the cancer-immunity balance: in this context, dysfunctional TLSs may even promote undesirable phenotypic changes in tumor cells. Consequently, CXCL13-CXCR5 axis, physiologically involved in immune cell recruitment and organization into a TLS, may foster immune-resistant/aggressive cancer cell phenotypes in a "hot" TME.

Aims

Objectives of the present proposal are:

1. Functional subclassification of intra-tumoral and peri-tumoral TLS in melanoma and Oral Squamous Cell Carcinoma (OSCC) and evaluation of their relationship with other immune cell subsets at the single cell level.
2. Investigation of TLS diversity in connection with cancer cell states at the single cell level.
3. Acquisition of novel knowledge about the intra-tumoral CXCL13/CXCR5 axis in these two tumor types with a particular focus on elucidating the impact of CXCL13 in antitumor immune response.

Experimental design

Melanoma and OSCC are chosen as tumor models expressing high levels of in situ CXCL13 and often characterized by ectopic TLS.

To reach our aims we will:

1. interrogate by means of sequential immunohistochemistry the immune landscape of melanoma and OSCC lesions receiving immunotherapy.
2. Use the fresh patient material to address the relationship between TLS signatures and cancer cell states at the single cell level.
3. Use the data collected ex vivo to integrate advanced oncology 3D models for the analysis of the CXCL13 effect of tumor cells.
4. Seek out in the intact tissue architecture those antigens and molecules potentially defining CXCL13-

associated immune-resistant tumor subpopulations expanded in "hot" TME.

Expected results

The exploration of the functional features and role of TLSs in cancer immunity is expected to uncover new potential targets for personalized cancer immunotherapy across different tumors. The CXCL13-CXCR5 axis could be usefully exploited to revert a dysfunctional TME; however, this work could also identify anti-PD1-resistant subpopulations candidates for antigen-specific immunotherapy (e.g. engineered T cells) or other approaches within combination strategies.

Impact on cancer

Although proposed in melanoma and OSCC, these findings could be easily translated to other tumor types. The novel functional classification of TLS and the CXCL13/CXCR5 axis elucidation may lead to rapid translation of our findings into the clinic for the benefit of cancer patients, as well as value creation.

Toma Tebaldi **MFAG - 2020**

Università degli Studi di Trento

Multimodal single-cell analysis of hematologic malignancies

Background

Cancer is a disease intrinsically entangled with complexity. The discovery of novel effective cures requires to deal with this complexity and greatly benefits from the development of high-resolution methods of investigation: genome-wide, multi-modal, single cell, spatially resolved. Understanding cancer at single-cell resolution within its architectural context is a scientific challenge requiring dedicated computational analysis dealing with the volume and sparsity of the data.

Hypothesis

N6-methyladenosine (m6A) is the most abundant RNA modification and plays key roles in RNA metabolism. Several proteins involved in m6A are essential in maintaining hematologic stem cell identity and they are strongly dysregulated in hematologic malignancies such as myelodysplastic syndromes, leukemia and multiple myeloma. The RNA epitranscriptome promises to be an important target for next-generation cancer treatments.

Aims

The aim of the proposed grant is to establish in Italy a computational biology lab focused on the study of hematologic malignancies by using cutting edge analysis of high-resolution high-throughput data.
I will develop two interconnected research lines involving
1) multi-modal analysis of single cell data to study the role of RNA modifications (the epitranscriptome) in hematologic malignancies and its relationship with the epigenome
2) analysis of spatially resolved genome-wide data to understand how the molecular architecture of gene expression in hematologic solid tumors can be used to develop effective cures.

Experimental design

I will profile in parallel gene expression changes (by single cell RNA-Seq) and epigenetic alterations (chromatin accessibility, by single cell ATAC-Seq, Assay for Transposase Accessible Chromatin) in hematopoietic progenitors upon loss of m6A.
I will develop dedicated computational strategies to integrate multiple single cell information layers and identify genes responsible for the association between m6A function and epigenetic changes.
I will identify genes and pathway affected by m6A loss and leading to aberrant hematopoietic differentiation.
I will explore the epitranscriptome/epigenome connection in human disease models, starting with the analysis of public datasets of hematologic malignancies and validating results in patient primary samples. I will test the combinatory effect of Mettl3 and PRC2 inhibitors in a mouse model of AML and in patient-derived xenografts.

Expected results

'- Characterisation of a novel intersection between the m6A epitranscriptome and the epigenome with single

cell resolution.
- Identification of pathologically relevant genes and pathways epigenetically affected by m6A loss and altering physiological hematopoietic cell proliferation and differentiation.
- Development of computational strategies and pipelines to integrate multiple layers of single cell next generation sequencing
data, coupled with spatially resolved gene expression analysis.

Impact on cancer

Dysregulation of PRC2 mediated transcriptional regulation has been extensively described in cancer and data is also emerging
about the role of m6A methylation in cancer. METTL3 and several proteins involved in m6A are strongly upregulated in
hematologic malignancies. The RNA epitranscriptome promises to be the next target for the treatment of cancer and other
disorders.
The characterization of a link between the m6A
epitranscriptome and the epigenome, responsible for altering normal hematopoiesis, would be groundbreaking, both seeding a new paradigm in basic biology and opening a broad range of therapeutic opportunities for translational research, within hematologic cancer and beyond.

Sarah Tettamanti **MFAG - 2024**

Fondazione M. Tettamanti M. De Marchi Onlus

MetaboCAR T cells: Rewiring Metabolism to Boost the Living Drug of the Century

Background

Chimeric antigen receptor (CAR) T-cell therapy has revolutionized cancer treatment, particularly for B-cell hematological malignancies and multiple myeloma, leading FDA and EMA approval of six CAR T-cell products. In the attempt of expanding CAR T-cell therapy to other cancers, such as Acute Myeloid Leukemia (AML) and solid tumors, challenges have been encountered, such as limited persistence and efficacy of CAR T cells due to immunosuppressive metabolic shifts and inhibitory signals in the tumor microenvironment (TME). Consequently, no CAR T products are approved for these indications.

Hypothesis

CAR T is a "living drug" that competes with tumor cells for essential nutrients in the TME while being affected by immunosuppressive metabolites derived from altered tumor cell metabolism. Thus, optimizing CAR T cells' fitness and bioenergetic metabolism is crucial for their effector functions, beyond merely selecting the optimal CAR. Therefore, I hypothesized that achieving metabolically fit CAR T cells could enhance prolonged persistence and potency in vivo, especially in the context of the known challenging TME of AML.

Aims

This research aims to better understand the CAR T-cell bioenergetic metabolism, including fine-tuning of nutrients supplementation. This knowledge will be fundamental for CAR T cells' immunometabolic rewiring to boost their clinical fitness and efficacy in a disease-specific context.

Experimental design

Using our proprietary non-viral Sleeping Beauty transposon platform, we will genetically manipulate Cytokine-Induced Killer (CIK) cells to express CARs. This platform was validated in a Phase I/IIa trial for CARCIK-CD19 in Acute Lymphoblastic Leukemia patients. We also developed next-generation Dual CD123/CD33 Split-CAR for AML to improve safety and efficacy.

To prove my hypothesis, we will:

WP1) Optimize CARCIK cell culturing conditions, to promote stemness for better adaptation to the harsh metabolic conditions of the TME.

WP2) Characterize immunometabolic fitness of diverse cell-sources (autologous vs allogeneic; peripheral vs. cord blood) and identify key metabolic features to enhance CARCIK effectiveness.

WP3) Identify nutrients and metabolites that boost CARCIK within the complex metabolic AML networks; and engineer CARCIK to target AML-specific metabolic pathways.

We will investigate the impact of these interventions on CARCIK immunometabolism and effector functions through long-term in vitro assays, including real-time imaging, protein (CyTOF), transcriptomic analyses (scRNAseq), and Seahorse metabolic assays. Anti-leukemic efficacy will be tested in immunodeficient NSG mice with AML cell lines and patient-derived xenografts, and safety will be assessed in humanized mice.

Expected results

This project will improve our understanding of the bioenergetic mechanisms governing CARCIK as living drugs and inform future next-generation CAR designs. Rewiring CARCIK metabolism to boost the antitumor potency is context-dependent and it will allow to develop personalized targeted therapies for solid and hematological malignancies.

Impact on cancer

By pioneering the development of metabolically fit CARCIK (MetaboCAR), our project aims to significantly prolong CARCIK persistence and enhance therapeutic outcomes. The anticipated impact includes a higher success rate in achieving long-lasting remissions, thereby improving the quality of life and survival rates for patients with difficult-to-treat cancers. This work will pave the way for the design of a clinical trial exploiting the non-viral CARCIK cell platform featuring optimized CARCIK and use of allogeneic sources as "off-the-shelf" therapy.

Gabriele Todisco **MFAG - 2024**

Università Humanitas

Dissecting dysfunctional immune response in patients with chronic myeloid neoplasms and unexplained inflammation.

Background

Recent description of Vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome provides substantial input to research focusing on immunologic consequences of clonal hematopoiesis, but still a coherent picture is lacking. Indeed, VEXAS syndrome constitutes a paradigmatic molecular model of acquired hemato-inflammatory syndrome secondary to clonal hematopoiesis driven by acquired UBA1 dysfunction. However, only 12% of myelodysplastic neoplasms (MDS) with history of unexplained chronic inflammation are harboring UBA1 mutation, suggesting the possibility of a causative interaction between clonal myeloid cells and primary chronic inflammation outside VEXAS diagnostic criteria. In addition, recent studies found that chronic inflammation enhances the fitness advantage of mutated hemopoietic stem cells, which suggests that - at least in specific genetic context - chronic inflammation also contributes to clonal progression rather than being a mere epiphenomenon.

Hypothesis

We hypothesize that unexplained chronic inflammation may represent a clinical phenotype of clonal hematopoiesis, either in the context of overt myeloid neoplasms or clonal hematopoiesis of undetermined potential (CHIP), with underlying molecular drivers. In this scenario, VEXAS syndrome may represent the tip of the iceberg of a much more complex condition with large disease borders and complex genetic landscape.

Aims

The project aims are summarized as follows:

1. To identify the molecular drivers accounting for primary autoinflammation in individuals with CHIP or MDS tested negative for UBA1 mutations
2. To provide a comprehensive evaluation of immunological landscape in the context of clonal hematopoiesis and unexplained chronic inflammation through integration of multi-layer single-cell omics data
3. To explore the relationship between impaired immunologic response and gut microbiome
4. To establish a multi-omics dataset to allow in silico drug screening and to provide clinical rationale for innovative therapeutic strategies.

Experimental design

This is a cross-sectional non-interventional clinical study focusing on patients with VEXAS syndrome (i.e. UBA1mutated) and symptomatic unexplained chronic inflammation co-occurring with CHIP or MDS (i.e. UBA1wildtype). Whole exome sequencing will be used to characterize clonal hematopoiesis drivers, whereas shot-gun metagenomics will be used for gut microbiota taxonomic profiling. Specific disease subgroups will be evaluated by multi-omics single cell approach to study the clonal phylogeny and the consequences of clonal hematopoiesis on innate and adaptive immunity.

Expected results

Based on mutation prevalence in CHIP/MDS and our preliminary data, at least one new gene of interest carrying somatic mutation is expected to be discovered and account for unexplained auto-inflammation. Single-cell multi-omic analysis applied to the resulting genetic classification will assess phylogeny and functional consequences of clonal hematopoiesis on hemopoietic cells. Results from this study will contribute to elucidate the relationship between gut microbiota and chronic inflammation, and potentially identify the basis for innovative therapeutic strategies.

Impact on cancer

The cellular and molecular mechanisms accounting for unexplained chronic inflammation in patients with clonal hematopoiesis and myeloid neoplasms are unknown. In these patients, chronic unexplained inflammation is associated with transfusion-dependent anemia, increased cardiovascular risk and reduced life expectancy. Currently, effective treatments are urgently needed for these patients. Full characterization of hemato-inflammatory syndrome pathobiology will provide innovative molecular targets for personalized treatment development capable of changing the natural history of myeloid neoplasms and related disorders.

Giacomo Volpe

Istituto Tumori "Giovanni Paolo II" I.R.C.C.S. Ospedale Oncologico di Bari

Unravelling the molecular roadmap to CEBPA-mutant driven leukemogenesis

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Background

Acute myeloid leukaemia (AML) is one of deadliest hematological neoplasms in which sequential mutations occur in progenitor cells and drive their leukaemic transformation. Ten to fifteen percent of de novo AMLs are characterized by mutations of the CEBPA gene, an essential transcription factor for haematopoietic stem cell self-renewal and granulocytic commitment. CEBPA mutations consist of either frameshift Nter mutations leading to selective loss of the p42 isoform or Cter in-frame insertion/deletions of the basic region/leucine zipper domain. To date, how sequential acquisition of CEBPA mutations rewires the epigenome to instructs myeloid transformation and drives clonal disease establishment is yet to be fully elucidated. A major obstacle in this endeavour, is the lack of clinically relevant disease models to study disease establishment and progression.

Hypothesis

Our proposal stems from the concept that CEBPA mutations activate several epigenomic switchers affecting haematopoietic homeostasis in terms of self-renewal and myeloid differentiation. We believe that a detailed molecular and functional catalogation of the main genetic dependencies associated to CEBPA- mutations will clarify unknown mechanisms of leukemogenesis and provide more rationally druggable targets in these AML settings.

Aims

We aim at generating a panel of CEBPA-mutant isogenic hiPSC lines to study their molecular and phenotypic consequences in vitro and in vivo. Our goals are:

i)Determining the mechanisms by which the acquisition of CEBPA mutations affect myeloid differentiation kinetics and instructs a leukemic phenotype in vitro;

ii)Tracking the dynamics of disease establishment and propagation in vivo;

iii)Identifying critical genetic dependencies and druggable molecular switchers in CEBPA-mutant leukaemic cells.

Experimental design

The research activity will be organized in the following tasks:

•Transfecting hiPSCs with constructs harboring either Nter or Cter CEBPA mutations, followed by collection and validation of hiPSC colonies for genotype, pluripotency and karyotype.

•Induction of haematopoietic differentiation of mutant hiPSCs into haematopoietic progenitor cells to determine how the mutation status influence the kinetics of differentiation, followed by liquid culture to induce terminal myeloid maturation. We will collect cells at d0, d4 and d7 of the differentiation and perform a comprehensive epitranscriptomic characterization by scRNA-seq and scATAC-seq.

•Transplantation of haematopoietic progenitors into immunocompromised mice and assessment of disease evolution through periodic tail bleeding.

•CRISPR/Cas9 drop-out screening in hiPSC-derived progenitor cells to identify novel mutation-associated genetic dependencies

Expected results

We believe that our proposed plan will produce crucial information in determining how CEBPA mutations impair haematopoietic homeostasis to drive leukaemogenesis. Our preliminary data show that our system recapitulates the typical phenotypic consequences of Cter mutations. We expect to dissect mutant-specific molecular programs and the transcriptional circuitries dictating oncogenic programs and to delineate the disease transformation roadmap in vivo, Finally we expect to identify novel genetic dependencies that will certainly highlight novel druggable vulnerabilities in CEBPA-driven AML.

Impact on cancer

This project will provide novel insights in the biology of CEBPA-driven leukemias, by means of an unprecedented system that recapitulates the leukemic cells behaviour in vitro and in vivo. We expect to highlight novel druggable vulnerabilities in CEBPA-driven AML, prompting the development of new targeted drugs to improve the AML treatment.

Stefania Volpe **Next Gen Clinician Scientist - 2023**

Istituto Europeo di Oncologia I.R.C.C.S. S.r.l.

Mondrian: multi-omics integrative modelling for stereotactic body radiotherapy in early-stage non-small cell lung cancer

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Background

While diagnostic anticipation has contributed to reduce mortality related to non-small cell lung cancer (NSCLC), it still ranks as first among big killers in Oncology. Treatment strategies for Early-stage (ES) disease are surgery or stereotactic body radiation therapy (SBRT), with successful local control rates for both approaches. However, regional and distant failure remain critical in SBRT, and it is paramount to identify predictive factors of response in this clinical setting to identify high-risk patients who may benefit from more aggressive approaches.

Hypothesis

Recently, a better understanding of NSCLC immunobiology has led to the successful use of immune checkpoint inhibitors in locally-advanced and metastatic disease. Therefore, there is increasing interest in translating this approach to ES disease. To date, ongoing investigations have enrolled surgical candidates, and a focus on SBRT is lacking. However, given the well-recognized immunomodulatory ability of SBRT, its combination with immune-checkpoint inhibitors is promising, and could substantially improve the outcomes of poor SBRT responders.

Aims

Given available preliminary evidence from the individual fields of radiomics, genomics and proteomics, the primary endpoint of MONDRIAN is to identify multi-omic biomarkers of SBRT response through advanced computational integration of the above-mentioned information layers. Secondary endpoints include the assessment of novel omic-derived prognostic factors, the design and validation of methodological radiomic and dosomic studies, and the longitudinal validation of gene expression and proteomics profiling between tissue- and liquid biopsy- derived samples.

Experimental design

MONDRIAN is designed as a prospective observational explorative cohort clinical study, with data-driven, bottom-up approach. It is expected to enroll 100 ES-NSCLC SBRT candidates treated at an Italian tertiary cancer center with well-recognized expertise in high-precision RT. To identify predictors specific to SBRT, MONDRIAN will include, and collect data from, patients treated with surgery in a 1:2 ratio, thus achieving a number of 200 operated subjects with comparable clinical characteristics. The project will have an overall duration of 60 months, and will be structured into five main tasks namely, i. Clinical Study, ii. Imaging/ Radiomic Study, iii. Gene Expression Study, iv. Proteomic Study, v. Integrative Model Building.

Expected results

Thanks to its multi-disciplinary nature, MONDRIAN is expected to provide an unprecedented opportunity to characterize ES-NSCLC from a multi-omic and comprehensive perspective, and to elucidate which

parameters interact to determine radiosensitive and radioresistant phenotypes.

Impact on cancer

Other than contributing to a mechanistic understanding of the disease, the study will assist the identification of high-risk patients in a largely unexplored clinical setting. Ultimately, this would orient further clinical research efforts on the combination of SBRT and (neo)adjuvant systemic treatments, such as immunotherapy, with the perspective of improving oncological outcomes in this subset of patients.