CLEPIC | Clinical Epigenetics International Conference

# Abstract Book

CLEPIC 2026 is organised in collaboration between the Josep Carreras Leukaemia Research Institute and International Society for Molecular and Clinical Epigenetics (iSMOCLEP)

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## Badalona (Barcelona), Spain

Josep Carreras LEUKAEMIA Research Institute

isMOCLEP

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# Abstracts Invited Speakers

## Epigenetic mechanisms and therapy of immune system cancers

**Presenter:**
Ari Melnick

### Abstract:

B-cell lymphomas, the most common hematologic malignancies, are genetically defined by recurrent mutations in transcription factors and chromatin modifiers. Most arise from B cells undergoing the germinal center (GC) reaction, a dynamic process regulated by immune synapse interactions with T follicular helper (TFH) cells that direct differentiation into plasma cells or memory B cells. A hallmark of the GC reaction is extensive epigenetic and 3D genome architectural reprogramming, although the mechanisms governing these transitions remain poorly understood. Many lymphoma-initiating mutations disrupt or hijack these immune synapse–driven epigenetic programs. During the proliferative phase of the GC response, B cells transiently suppress immune synapse gene expression to permit somatic hypermutation, while renewed immune synapse signaling guides GC exit and terminal differentiation. Mutations in epigenetic regulators such as EZH2, CREBBP, and KMT2D disrupt these programs and remodel the tumor immune microenvironment. Among these, ARID1A, a component of the BAF chromatin remodeling complex, is frequently mutated in B-cell lymphomas. We demonstrated that ARID1A controls GC B-cell fate by enabling sequential and cooperative binding of PU.1 and NF-κB at genes required for cytokine and CD40 signaling downstream of TFH interactions. This mechanism explains GC-specific NF-κB transcriptional programming. Loss of ARID1A skews GC output toward immature IgMκCD80κPDL2κ memory B cells with enhanced capacity to re-enter GC reactions. In mouse models, ARID1A haploinsufficiency cooperates with BCL2 to accelerate aggressive lymphoma progression. Clinically, ARID1A-mutant lymphomas exhibit a memory B-cell–like transcriptional state associated with poor prognosis and increased transformation risk. Other lymphoma mutations act through distinct mechanisms. SETD2 mutations impair DNA damage recognition and promote genomic instability, whereas Histone H1 mutations reactivate embryonic stem cell–like transcriptional programs, suggesting that increased epigenetic plasticity enhances tumor fitness. Together, these findings support a unifying model in which disruption of tightly regulated GC epigenetic and immune synapse programs drives lymphomagenesis. Importantly, many of these alterations are therapeutically targetable, and our mechanistic insights have enabled development of precision immuno-epigenetic therapies that are showing promising clinical activity in patients.

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# Sustained epigenetic editing to control transcriptional heterogeneity

**Presenter:**
Pernette J Verschure

## Abstract:

Background: Cell identity is largely maintained through epigenetic gene regulation, yet even genetically identical cells can display substantial variability in transcriptional activity and treatment responsiveness. Understanding how transcriptional heterogeneity influences epigenetic reprogramming and vice versa, may provide important insights into the development of therapy resistance in estrogen receptor positive (ER+) breast cancer. We investigate how epigenetic therapies modulate transcriptional dynamics and heterogeneity in MCF7 breast cancer cells, exploring the relationship between gene permissiveness to epigenetic editing and transcriptional responses at single cell resolution.
Methods: MCF-7 cells were treated with a panel of epigenetic drugs and selective estrogen degrader fulvestrant. Single-molecule RNA fluorescence in situ hybridization (smRNA-FISH) was used to quantify transcriptional activity at single-cell and single transcript resolution for the estrogen-responsive genes AREG and GREB1, stemness- and plasticity-associated genes CD24 and CD44, and housekeeping gene PGK1. Single-cell transcript distributions were used to calculate mean expression levels, burst size, burst frequency and Fano factors, enabling the assessment of treatment induced changes in transcriptional output and heterogeneity. To evaluate gene permissiveness to epigenetic editing, we use a 'hit-and-run' strategy involving transient epigenetic perturbation followed by longitudinal single cell transcriptional analyses. A library of fluorescently tagged CRISPR/dCas-based epigenetic editors was generated by fusing diverse chromatin-modifying enzymatic domains to dCas9. Their activity was assessed using reporter cell lines engineered to represent distinct chromatin environments and targeted with single or multiple guide RNAs. Chromatin states were defined using Hidden Markov Modeling-based integration of multiple histone modification datasets. The efficacy of selected epigenetic editor combinations will be evaluated through CRISPR/dCas epigenetic perturbation combined with single cell RNAseq as readout, targeting these genes associated with specific chromatin states.
Results and Conclusions: Treatment with epigenetic and endocrine therapies induced distinct, gene-specific alterations in transcriptional activity and heterogeneity. These differential transcriptional responses provide valuable insights into cellular permissiveness to epigenetic reprogramming and how chromatin context influences treatment outcomes. Our findings highlight the importance of single-cell transcriptional dynamics in understanding epigenetic regulation during the acquisition of treatment resistance and open-up new possibilities for precision medicine in ER+ breast cancer.

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# Network Medicine and the Promise of Epigenetics

**Presenter:**
Dawn L. Demeo

## Abstract:

Network medicine is an emerging field that applies systems biology, computational modeling, and molecular medicine to understand complex human diseases as the product of interconnected biological networks, rather than isolated genes or single pathways. Past approaches have focused on genetic variation and discrete molecular targets; however, complex diseases such as asthma, diabetes, cardiovascular disease, and dementia arise through dynamic interactions among genetic risk, epigenetic factors, proteins, metabolites, immune pathways, environmental exposures, and social determinants of health. Network medicine seeks to map these interactions into integrated disease networks, enabling insights through previously unrecognized relationships between "layers" of the human omes. Epigenetic variation offers a powerful mechanistic bridge between genetic predisposition, environmental influences, human health and disease. Epigenetic modifications, as an archive of environmental stimuli such as diet, pollution, stress, infection, aging, and other exposures, elevate epigenetics as highly relevant to consider in the context of precision medicine and population health. The integration of epigenetics into network medicine has significant implications for both diagnostics and therapeutics. Rather than viewing diseases as static or deterministic, network-based epigenetic models recognize that biological systems are adaptive and continuously reshaped by internal and external signals. Epigenetic signatures may therefore serve as early biomarkers of disease susceptibility, treatment response, or disease progression. Network medicine also enables the study of multimorbidity, which is increasingly common in aging populations. Shared epigenetic aging and inflammatory pathways may help explain why certain diseases cluster together over time, and why interventions targeting one pathway can influence multiple conditions simultaneously. Advances in artificial intelligence, multi-omics integration, and large-scale computational modeling are accelerating network medicine, allowing researchers to analyze biological complexity at an unprecedented scale and resolution. Despite the promise of network medicine and the inclusion of epigenetic marks, important challenges remain, including data standardization, the need for computational models that can handle epigenome-wide resolution, and clinical translational relevance. The convergence of network medicine and epigenetics represents a major paradigm shift. By moving beyond reductionist approaches toward a systems-level understanding of health and disease, this field has the potential to transform and 21st century precision medicine.

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# Form and Function: an exploration of 3D genome regulators

**Presenter:**
Elzo de Wit

## Abstract:

The organisation of the genome inside the nucleus plays an important role in DNA repair, DNA replication and gene expression regulation. Two key players in 3D genome organisation are CTCF and the cohesin complex. The latter act as a DNA motor to create loops in the DNA in a process known as extrusion. CTCF on the other hand acts as a barrier to loop extrusion leading to formation of chromatin loops. While cohesin and CTCF are essential in organismal development and in cells, their acute depletion has relatively limited consequences for gene expression. To investigate the role of CTCF during development we used a mouse embryonic stem cell (mESC) line from which we could acutely deplete CTCF to investigate the consequences for in vitro development. We find that CTCF is not required for differentiation, but is crucial for morphogenesis. In the early stages of development a looping independent function is important for development, whereas later in development the looping function also becomes important. I will present data that explores the promoter-regulatory role of CTCF in gene expression.

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# Synergy among enhancers involves an increase in transcriptionally productive enhancer-promoter contact

**Presenter:**
Álvaro Rada-Iglesias

**Abstract:**

Enhancers are non-coding cis-regulatory elements that control the expression of distally located genes in a tissue- and time-specific manner. Recent studies indicate that enhancers can differ in their underlying genetic architecture and regulatory properties. However, these different types of enhancers were previously investigated under rather variable conditions (e.g. model organism, cell type, enhancer-promoter distance, type of target promoter, etc.), thus introducing confounding factors that make it difficult to discern the distinct regulatory properties of each enhancer type. To overcome these limitations, here we generated transgenic mouse embryonic stem cells (mESC) lines in which different types of synthetic enhancers (i.e. “typical” enhancer, CTCF-associated enhancer, enhancer cluster/super-enhancer) were built upon the same “core” neural enhancer and inserted at the same distance (i.e. 100 Kb) from a typical developmental gene (i.e. Gata6). Subsequently, the mESC lines were differentiated to systematically compare the regulatory properties of the different enhancer types under identical conditions. Regarding the CTCF-associated enhancer, our data revealed that the addition of a CTCF site to the “core” enhancer increased insulation and led to the formation of a smaller contact domain, while having a rather mild effect on enhancer-promoter contact frequency and target gene expression. On the other hand, in comparison to the “core” enhancer alone, the enhancer cluster synergistically increased target gene expression and burst fraction. Importantly, we found that, in contrast to previous models, the strong regulatory activity of the enhancer cluster cannot be explained by changes in enhancer-promoter contact frequency or the formation of transcriptional condensates. Instead, our data suggest that the emergent regulatory properties of enhancer clustering preferentially entail an increase in RNA Polymerase II pause release and, thus, in the fraction of enhancer-promoter contacts that are transcriptionally productive.

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# Strategies, challenges and outlook of epigenetic research in plants

**Presenter:**
Jason Gardiner

## Abstract:

In plants, epigenetics is a key component of transcriptional regulation, crucial for normal development and environmental responses. The mitotic and meiotic heritability of epigenetic marks, such as DNA methylation, positions plant epigenetics as a vital tool for agriculture, expanding the toolbox beyond merely selecting or editing nucleotide sequences. Understanding how different epigenetic marks selectively regulate specific processes provides insight into how we can reprogram plant development and responses. As new and improved systems for chromatin engineering continually emerge, it is necessary to develop a diverse toolbox of strategies to tackle unique challenges. This talk outlines current strategies and challenges for non-targeted and targeted chromatin engineering, enabling manipulation of the transcriptome. It also offers a brief outlook on potential future directions in plant epigenetic research.

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# Detecting DNA methylation changes separating humans from nonhuman African apes

**Presenter:**
Liran Carmel

## Abstract:

Human lineages, including anatomically modern and archaic ones (Neanderthals and Denisovans), show notable morphological and behavioral differences from nonhuman African apes like chimpanzees and gorillas. As regulatory changes are thought to be a major driver of phenotypic divergence, we sought to identify such regulatory changes along the lineage leading to humans since the split from chimpanzees. Given that DNA methylation is the best proxy for gene expression in ancient samples, we compared methylation maps across modern humans and nonhuman African great apes, alongside DNA methylation reconstructed from ancient DNA of anatomically modern humans (AMHs) and archaic humans.
To this end, we experimentally generated whole-genome DNA methylation maps from bones of a gorilla, a chimpanzee, and six present-day humans, and supplemented these with reconstructed methylation profiles from a Neanderthal, a Denisovan, and 28 high-coverage AMH samples. Applying stringent and conservative filtering criteria, we detected 270 DMRs distinguishing humans from nonhuman African apes, as well as a similar number showing chimpanzee-specific methylation. Together, this constitutes the most comprehensive resource on changes in DNA methylation across apes. These DMRs tend to be associated with facial morphology, bone remodeling and heart rate control. Notably, we observe across multiple tissues a strong signal of differential regulation in genes related to hair follicles and sweat glands, traits that are distinctly different between humans and apes.

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# Genetic impacts on DNA methylation variance capture gene environment interactions and cell-type effects

**Presenter:**
Jordana Bell

## Abstract:

Characterising genetic impacts on DNA methylation can improve our understanding of the pathways that underlie gene regulation, as well as processes involved in human development, disease, and ageing. Following an overview of recent large-scale systematic studies of genetic effects on the human blood methylome (Min et al. 2021, Nature Genetics; Villicaña et al. 2023, Genome Biology), this work will explore extensions to identify gene–environment interaction effects. Gene–environment interactions can lead to differential phenotypic variability across genotype groups. Therefore, genetic variants that interact with environmental exposures can show associations with phenotypic variability, or variance quantitative trait loci (vQTLs). Although changes in DNA methylation variability have been observed in several diseases, vQTLs for methylation levels (meQTL) have not yet been explored in depth. This work will present findings of novel genetic effects on human DNA methylation variability by applying a unique monozygotic twin study design (Zhang et al. 2026, Genome Biology). Discussion will extend to ongoing work aiming to identify vmeQTLs in large-scale population-based studies within the framework of the Genetics of DNA Methylation Consortium (GoDMC, https://www.godmc.org.uk/). The results show the potential of vmeQTLs to identify gene–environment interaction effects on the human blood methylome and provide novel insights into complex traits.

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# Programming the Epigenome: from tools to discovery

**Presenter:**
Jamie Hackett

## Abstract:

Chromatin modifications are tightly associated with gene expression programs that shape development and disease trajectories. Yet their causal roles - and the regulatory logic by which the epigenome operates - remain elusive, highly context-dependent, and incompletely understood. Here, I will discuss our efforts to move beyond correlation and interrogate what the epigenome actually does - and how we can manipulate it for therapeutic applications. By building programmable epigenome editing tools that enable precision changes to chromatin states, we have attempted to define the causal relevance of epigenetic modifications in transcriptional control, and probe context-dependent interactions across cell types and disease. Such epigenome editing technologies also hold great promise as a therapeutic modality to calibrate gene activity, and I will cover recent updates.

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# Epigenomic signatures of neurodegenerative diseases

**Presenter:**
Katie Lunnon

## Abstract:

Many neurodegenerative diseases that cause dementia are multi-factorial with the exact aetiology still unknown. Genetic and epidemiological studies have highlighted both genetic and modifiable lifestyle risk factors, with epigenetic processes suggested to mediate their interaction. DNA methylation is the best characterised epigenetic mechanism in neurodegenerative diseases, particularly in Alzheimer's disease. Over the last decade several epigenome-wide association studies (EWAS) of post-mortem brain tissue, including our own, have highlighted replicable differentially methylated sites in the genome associated with pathology, with our subsequent meta-analyses reporting hundreds of loci. More recently we have performed EWAS and meta-analyses in Lewy body dementia, nominating Lewy body pathology associated loci, including those driven by methylation quantitative trait loci in the SNCA locus. Interestingly, when we compared brain epigenetic signatures between different neurodegenerative diseases we observed a shared methylation signature in cortex, although the most robust loci are disease specific.

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# Targeting reversible histone acetylation and methylation

**Presenter:**
Manfred Jung

## Abstract:

Reversible lysine methylation in histone proteins is one of the major mechanisms of epigenetic regulation and also occurs on non-histone substrates. Methyltransferases introduce this epigenetic mark, demethylases remove the methyl groups, and proteins containing methyl-lysine binding domains recognize methylated lysines and propagate the epigenetic signal. Here, we present the structure-based discovery of inhibitors targeting the lysine methyltransferase KMT9, as well as inhibitors of the double chromodomain of the ATP-dependent chromatin remodeler CHD1. Starting from weak and rather unselective hits, optimized inhibitors for these two targets were developed through iterative cycles of structural analysis, synthesis, and biological testing. These compounds show high in vitro potency, engage their targets in cell culture, and exhibit antiproliferative properties in cultured cancer cells. These inhibitors can now be used to study the functions of KMT9 and CHD1 and to further evaluate their therapeutic potential.

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# The expanding biochemistry of JmjC histone lysine demethylases

**Presenter:**
Akane Kawamura

## Abstract:

Post translational modifications (PTMs) on histone proteins play a central role in shaping chromatin structure and regulating transcription. Studies on PTMs, such as acetylation, methylation, and phosphorylation, have revealed how a coordinated network of writer, eraser, and reader proteins establishes and interprets chromatin states. New histone PTMs continue to emerge, and many associated epigenetic enzymes and chromatin associated factors have been identified, emphasising the increasing complexity of the histone code.

Histone lysine methylation is one of the best characterised PTMs and is dynamically regulated by lysine methyltransferases and demethylases.[1] Among the erasers, the Jumonji C lysine demethylases (JmjC-KDMs) comprise a major family of histone demethylases and belong to the wider Fe(II)/2-oxoglutarate-dependent oxygenase superfamily.[2] Since their discovery as histone lysine demethylases 20 years ago, JmjC-KDMs have been shown to act on a broader set of substrates and to catalyse reactions beyond classical N-methyl lysine demethylation, including N-methyl arginine demethylation.[2-4]

In this talk, I will discuss our recent biochemical studies defining the substrate scope and mechanistic features of JmjC-KDMs, highlighting their substantial catalytic flexibility and emerging ‘writing’ capabilities. [5-7] We demonstrate the importance of sequence context in determining the relative efficiencies of different JmjC-KDM-catalysed reactions, and identify unexpected, stable oxidative histone modification products. These oxygen-dependent reactions expand the chemical diversity of the histone code and may have physiological relevance.

Together, these findings broaden the functional repertoire of JmjC-KDMs and suggest wider roles in chromatin regulation, development, homeostasis, and disease.

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# Exploiting the cell-of-origin fingerprint for methylation-based tumor classification

**Presenter:**
Stefan M. Pfister

## Abstract:

DNA methylation patterns preserve a stable epigenetic record of a cell's developmental origin and differentiation history, providing a molecular "fingerprint" that can be leveraged to identify and classify human cancers. By exploiting this cell-of-origin signal, researchers and clinicians at the German Cancer Research Center (DKFZ) and Heidelberg University Hospital (UKHD), together with many collaborators worldwide, have transformed brain tumor diagnostics (and beyond) from a largely morphology-based discipline into a precise, data-driven approach. This work culminated in the Heidelberg Brain Tumor Classifier, an artificial intelligence-based platform capable of distinguishing more than 150 molecularly defined brain tumor entities based on their methylation signatures. Freely accessible to the international research community, the classifier has now analyzed more than 180,000 tumor samples worldwide and contributed to the discovery of many previously unrecognized tumor types. Multiple international validation studies, including a landmark population-based study of more than 1,200 pediatric brain tumor patients, demonstrated that methylation profiling refines or revises diagnoses in up to one-third of cases, directly impacting treatment decisions and patient outcomes. The clinical significance of this cell-of-origin-based approach was recognized through the incorporation of methylation profiling as a recommended diagnostic criterion for many brain tumor entities in the 2021 WHO Classification of Central Nervous System Tumors. Translation into routine healthcare has been accelerated through Heidelberg Epignostix GmbH, a DKFZ/UKHD spin-off developing certified AI solutions for precision oncology and driving international clinical implementation, including reimbursement approval in the United States and other countries. Beyond technological innovation, this work has established a model for equitable global healthcare. Through the MNP Outreach Consortium (www.mnp-outreach.com), advanced molecular diagnostics are being implemented in low- and middle-income countries through training, infrastructure development, cloud-based analytics, and international molecular tumor boards. In parallel, studies of underrepresented populations and ethnicity-associated methylation signatures are providing new insights into global differences in brain tumor incidence and biology. Together, these achievements demonstrate how epigenetic fingerprints can be harnessed to advance cancer diagnosis, precision medicine, and global health.

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# Targeting RNA modifications in blood malignancies

**Presenter:**
Kamil Kranc

**Abstract:**

N/A

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# An abundant chromatin-binder confers transcriptional addiction to

**Presenter:**
Akis Papantonis

**Abstract:**

N/A

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# Chromatin regulation and cancer

**Presenter:**
Yang Shi

**Abstract:**

N/A


# Clinical Epigenetics International Conference 2026

## Only Poster

### 1. Clinical cancer epigenetics

ABS-2198 Dissecting METTL3 Inhibition as a Potential Platform for Synthetic Lethality in NSCLC

ABS-2261 Dissecting the epigenomic and transcriptomic landscapes of pediatric acute leukemia using multi-modal single-cell sequencing

ABS-2213 Establishing METTL16 as a key epigenetic and transcriptional regulator in GBM carcinogenesis

ABS-2275 Exploring the epigenetic mechanisms underlying the evolution of CRC drug-tolerant persister cells

ABS-2202 Functional characterization of CBX2 inhibition in Glioblastoma

ABS-2191 Investigating the effect of decitabine on the transcriptome of breast cancer cell lines.

ABS-2172 Melanomas with mutations in chromatin-regulating genes are positively associated with mutational burden and improved response to checkpoint immunotherapy.

### 2. Epigenetic technologies

ABS-2268 Development of the tool for identification of phase specific epigenetic maintenance profile in healthy and tumor cells at single-cell resolution

ABS-2173 High-Throughput, Multiplexed, Quantitative Epigenetic Profiling of Cells, Tissues, and Liquid Biopsies

ABS-2231 The 6-base genome unlocks novel mechanistic biomarkers and enhanced biological insight from challenging clinical samples

ABS-2183 TIP-ChIP: Tagmented, Indexed, and Pooled ChIP-Seq

ABS-2269 Variable gene regulatory responses to epigenetic editing across distinct epigenetic chromatin contexts

### 3. Epigenetic mechanisms in human disease

ABS-2248 CBX6 as an epigenetic regulator of differentiation in Glioblastoma

ABS-2223 DNA Methylation Reconfiguration in Temozolomide-Resistant Recurrent Glioblastoma: a Comparison between epigenetic dynamics in vitro and in vivo

ABS-2270 Epigenetic Editing–Mediated Regulation of PPARGC1A to reprogram Mitochondrial Function and Suppress Colon Cancer Progression

ABS-2219 Epigenomic signatures and accelerated epigenetic aging in major depressive disorder: preliminary results from the OPADE project

ABS-2249 Exploring the therapeutic potential of Histone Deacetylase 11 (HDAC11) in Diabetic Cardiomyopathy and Heart Failure

ABS-2229 HDAC7 restrains Diffuse Large B Cell Lymphoma progression and enhances immunochemotherapy response through upregulation of CD20

ABS-2207 Insights into the biological role of LAV-BPIFB4: epigenetic mechanisms and possible therapeutic implications

ABS-2245 LINC complex disruption impairs DNMT1 dynamics and nuclear lamina organization in RPE-1 cells

## 4. Epigenetic and chromatin in stemness, aging and development

ABS-2187 HAT4 regulates muscle integrity and function through epigenetic modulation of MEF2C in zebrafish

ABS-2218 Identification of a putative cis-regulatory element of the human NANOG gene in cancer stem cells

ABS-2265 Identifying Age-Associated DNA Methylation Sites and Their Functional Relevance Using Epigenetic Editing in Dermal Fibroblasts

## 5. Epigenetics in drug discovery and therapeutics

ABS-2230 Defining novel therapies to prevent lineage switch in B lymphocyte malignancies through HDAC7 induction

ABS-2220 Epigenetically Driven Variability in Therapeutic Response in PDAC

ABS-2260 Small molecule inhibition of NUP98::KDM5A eliminates leukemic dependencies inducing differentiation in AML models

## 7. Epigenetic biomarkers

ABS-2264 A plasma methylation model for tumor fraction estimation to deconfound epigenetic drivers of therapy resistance in metastatic colorectal cancer

ABS-2253 Advancing lung cancer detection: discovery and analytical validation of novel plasma ccfDNA methylation biomarkers

ABS-2192 CBX3 as a prognostic biomarker in lung cancer

ABS-2252 DNA methylation heterogeneity within multiple myeloma patients map to enhancers and repressed Polycomb regions and seems to be associated with survival outcome

ABS-2201 Epigenetic role of HDAC2 in colorectal tumorigenesis

## To be considered for an Oral Presentation

### 1. Clinical cancer epigenetics

ABS-2263 A Focused Library Screening Reveals Pan-PKC Inhibitors as Potent Sensitizers for ATRA Therapy in Non-APL AML

ABS-2258 CBX2 as a Potential Target for Therapeutic Intervention in CRC

ABS-2228 Decoding cellular complexity of the microenvironment of epigenetically driven pediatric high grade gliomas

ABS-2259 Differential genomic localization of H3 variants in the progression of gastric cancer.

ABS-2150 DNA methylation variability is a defining feature of tumor epigenomes with biological and prognostic significance

ABS-2235 Epigenetically associated CD146 heterogeneity defines hybrid epithelial–mesenchymal states in TNBC Cells

ABS-2217 Genome-wide Co-regulation Networks in Aberrant DNA Methylation of Acute Myeloid Leukemia

ABS-2241 Integrated single-cell genetic and epigenetic profiling reveals desynchronized subclonal evolution in juvenile myelomonocytic leukemia

ABS-2194 Investigating how cancer-specific enhancer rewiring shapes gene regulatory programmes in high-grade serous ovarian carcinoma

ABS-2176 Transposon Derived Neo-Antigens in NSD1 Mutant Head and Neck Cancers

ABS-2182 Validation of the WID-CIN methylation test for detection of Cervical Intraepithelial Neoplasia grade 3 or worse in an independent cohort

### 2. Epigenetic technologies

ABS-2196 Beyond bisulfite: Directly quantifying DNA methylation in the clinic

ABS-2227 Cell-specific DNA methylation in human alpha and beta cells regulates gene expression in type 2 diabetes

ABS-2242 FLEA-ChIP: A differentiated platform for challenging clinical samples

ABS-2179 The 12 o'clock assay: an optimized dodecaplex droplet digital PCR assay for improved DNA methylation quantification and epigenetic clock-based age-predictions

### 3. Epigenetic mechanisms in human disease

ABS-2250 Androgen stimulation rapidly reorganizes temporal 3D genome and epigenome states to trigger AR-mediated transcription in prostate cancer.

ABS-2224 Biomolecular condensation of E2A-PBX1 oncoprotein drives chromatin organization and leukemogenesis in B-ALL

ABS-2236 Chromatin dysregulation in uterine leiomyomas: insights from CRISPR engineered model cell lines

ABS-2257 Context-dependent recruitment of co-factors directs RUNX1 activity in normal and leukemic cells

ABS-2149 Dialysis-Associated Epigenetic DNA Methylation Signatures Linked to Inflammation in Hemodialysis Patients

ABS-2240 DNA methylation loss impairs nuclear architecture and promotes chromosomal instability through heterochromatin relaxation

ABS-2232 Dynamic changes of discordant methylation patterns at adjacent CpG sites are associated with pathogenesis of chronic lymphocytic leukemia

ABS-2243 Dynamic epigenetic regulation of BCLAF1 splicing in acute myeloid leukemia

ABS-2157 Epigenetic and Plasma C-Reactive Protein in Relation to Regional Brain Structure and Psychosis Outcomes

ABS-2237 Epigenetic Regulation of CD146 Reveals Phenotypic Plasticity and Stable Subclones in TNBC Cells.

ABS-2212 hsa-mir-98-5p – a new player in classic Hodgkin lymphoma development

ABS-2188 Hypoxia-driven lipid rewiring of microglia cells reveals a targetable epigenetic vulnerability in high-grade gliomas

ABS-2181 Identification of Parkinson's disease-associated regulatory variants in human dopaminergic neurons reveals modulators of SCARB2 and BAG3 expression

ABS-2225 LAT1-driven metabolic–epigenetic reprogramming underlying radiobiological resilience in glioblastoma: implications for BNCT

ABS-2185 Maternal mental health during pregnancy and placental DNA methylation

ABS-2221 Oral Pathogens Induce Trained-Like Immunity in Gingival Fibroblasts: Epigenetic Perspective on Stromal Memory in Periodontitis

ABS-2234 Single-cell multiome analysis reveals AP-1–driven regulatory reprogramming during endocrine therapy in ER positive breast cancer

ABS-2239 Transposable elements reorganise the 3D genome structure in CDK4/6 inhibitors resistant breast cancer

ABS-2171 Uncovering the role of epigenetics in LACTBs tumor suppressive landscape

## 4. Epigenetic and chromatin in stemness, aging and development

ABS-2210 A regulatory eRNA at the Nanog locus controls epigenetic stability and chromatin architecture in mouse embryonic stem cells

ABS-2195 Comparison of a novel microRNA clock with established DNA methylation clocks in determining gestational age.

ABS-2197 EpiDirect®-Clock: a rapid, cost-efficient, bisulfite-free, qPCR-based age prediction tool for forensic casework

ABS-2178 Fueling the Nucleus: Nuclear GDH1 Links Metabolism and Epigenetic Regulation to Stem Cell Identity.

## 5. Epigenetics in drug discovery and therapeutics

ABS-2211 "Writing and Erasing the Chromatin Code: KMT5a and p53 Cooperate to Control p62/SQSTM1 in Glioblastoma"

ABS-2190 CDK12/CDK13 inhibition disrupts transcriptional elongation and replication fork progression in glioblastoma

ABS-2208 DEVELOPMENT OF CHEMICAL PROBES FOR METHYL-LYSINE READER DOMAINS IN EPIGENETIC REGULATION

ABS-2244 HEPATOCYTE-SPECIFIC DNMT3A AND DNMT3B KNOCKOUT MICE IS LESS SENSITIVE ACETAMINOPHEN THAN WILD-TYPE

ABS-2215 Modulation of histone code to manipulate macrophage responses to P. gingivalis

ABS-2209 Optimizing T Cell Expansion: Mitigating Culture-Associated DNA Methylation Changes with Decitabine and Vitamin C

ABS-2203 Targeted epigenetic interventions enable next-generation T cell products for adoptive cell therapies.

ABS-2247 Treatment with epigenetic inhibitors restores cGAS–STING signalling in immunologically “cold” tumors

## 6. Epigenetics of the tree of life

ABS-2184 Beyond the CpG: Dissecting the functions of atypical DNA methylation during early vertebrate development

## 7. Epigenetic biomarkers

ABS-2226 Biomarker identification using DNA methylation signatures of patient-derived colorectal cancer tumoroids

ABS-2177 DNA methylation signatures of human adaptation to chronic hypobaric hypoxia

ABS-2222 Epigenomic signatures during disease evolution in Preclinical Systemic Sclerosis

ABS-2174 Lost in translation: How CpG site selection and assay design determine the clinical value of DNA methylation markers

ABS-2180 Plasma histone monomers as novel diagnostic markers in adult glioblastoma

ABS-2233 Preservation, Disruption, and Acquisition of Cell-Type-Specific DNA Methylation in Cancer

ABS-2186 Towards Non-Invasive Detection of Ferroptotic Organ Injury via DNA Methylation Profiling

# Dissecting METTL3 Inhibition as a Potential Platform for Synthetic Lethality in NSCLC

Only Poster | 1. Clinical cancer epigenetics

**Main author**: Dr. Maryam Gull (Department of Precision Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy · 1)

**Abstract**:
Background Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality, driven by recurrence, metastasis, and acquired therapy resistance. Single-agent treatments, including conventional chemotherapeutics, frequently fail due to tumor plasticity and adaptive resistance mechanisms. N6-methyladenosine ($m^6A$), the most abundant internal mRNA modification in eukaryotes, is dynamically regulated by writers, readers, and erasers and plays a key role in oncogenesis. In parallel, epigenetic modulation through histone deacetylase inhibitors (HDACi) has emerged as a strategy to enhance therapeutic response. Here, we systematically evaluated chemotherapeutic and epigenetic agents, alone and in combination with the METTL3 inhibitor STC-15. We identify rucaparib (PARPi) as potential partners for METTL3 inhibition and demonstrate synthetic lethality across genetically distinct NSCLC models. Methods NSCLC cell lines A549 and NCI-H1299 were treated with STC-15, cisplatin, doxorubicin, temozolomide (TMZ), paclitaxel, rucaparib, and SAHA as single agents or in combination. Cell viability was assessed using CCK-8, colony formation, and time-course proliferation assays. Bioinformatic analyses using DepMap and GEPIA were conducted to assess METTL3 dependency and its correlation with drug target expression in lung cancer datasets. Results STC-15 alone produced only a slight reduction in viability in NSCLC cells. Co-treatment with SAHA resulted in a moderate but consistent additional decrease in viability. Notably, Rucaparib demonstrated robust and reproducible synthetic lethality in combination with STC-15. In A549 and NCI-H1299 cells, this combination significantly reduced cell viability ($p$ 0.005, $n = 3$), with concordant effects observed in CFU and proliferation assays. Conclusions METTL3 inhibition represents a viable synthetic lethality platform in NSCLC. While the combination of METTL3 inhibition and epigenetic targeting yields modest but consistent effects, the strong synergy between METTL3 and PARP inhibition provides a compelling rationale for combined epitranscriptomic and DNA repair targeting. This strategy may help overcome resistance to single-agent therapies. Ongoing RNA-seq and pathway analyses, alongside epigenetic silencing of METTL3 in combination with PARP and HDAC targeting, will further define underlying mechanisms and support clinical translation.

↑ Beginning

# Dissecting the epigenomic and transcriptomic landscapes of pediatric acute leukemia using multi-modal single-cell sequencing

Only Poster | 1. Clinical cancer epigenetics

**Main author**: Dr. Mohanna Mohammadi (University of Campania - Luigi Vanvitelli · PhD student)

**Co-authors**:

- University of Campania - Luigi Vanvitelli: Dr. Ali Hossein Torkamaan Gholami (PhD student); Prof. Nunzio Del Gaudio (Assistant Professor); Prof. Lucia Altucci (Full Professor)
- Princesse Maxima Center for Pediatric Oncology: Dr. Cristoforo Grasso (PostDoc); Prof. Hendrik Gerard Stunnenberg (Research Group Leader)

**Abstract**:
Background: Relapse in acute myeloid leukemia (AML) remains a major clinical challenge, particularly in patients harboring alterations in the RAS signalling axis, including both canonical RAS mutations and upstream regulators. While these alterations suggest a shared oncogenic origin, the mechanisms driving relapse remain unclear. We hypothesized that persistent cellular programs, supported by epigenetic regulation, contribute to relapse. Methods: We performed single-cell RNA sequencing (scRNA-seq) and single-cell ATAC sequencing (scATAC-seq) on paired diagnosis (DX) and relapse (RE) samples from pediatric AML patients with RAS-pathway alterations. Cells were annotated using reference-based approaches. Non-negative matrix factorization (NMF) was applied to identify transcriptional programs from scRNA-seq data. Program activity was compared across conditions, cell types, and patients. Chromatin accessibility and transcription factor (TF) activity were assessed using scATAC-seq and chromVAR. Results: We identified two dominant and conserved transcriptional programs across patients: (i) a metabolic program and (ii) a stem/progenitor-like program associated with cytoskeletal and motility signaling. Both programs were present at diagnosis and persisted at relapse, indicating stable underlying cellular states. While their relative abundance shifted across cell types and patients, and conditions, these core programs were consistently maintained. Integration with scATAC-seq is being used to investigate the epigenetic mechanisms underlying the persistence of these programs, with preliminary analyses suggesting involvement of transcription factor networks such as NFY and AP-1/BATF. Conclusions: Relapse in RAS-driven AML is characterized by persistence and modulation of pre-existing cellular programs rather than the emergence of new states. Epigenetic maintenance of these programs may underlie treatment resistance, highlighting stable regulatory circuits as potential therapeutic targets. Funding • Princess Maxima Center for Pediatric Oncology, Utrecht, the Netherlands • PhD Program in Translational Medicine at University of Campania Luigi Vanvitelli.

↑ Beginning

# Establishing METTL16 as a key epigenetic and transcriptional regulator in GBM carcinogenesis

Only Poster | 1. Clinical cancer epigenetics

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- Department of Neurosurgery, Brigham and Women’s Hospital, 60 Fenwood Road, Boston, MA 02115, USA.: Prof. Pierpaolo Peruzzi (Full Professor)
- Department of Life Sciences, Health and Health Professions, Link Campus University, Via del Casale Di San Pio V 44, Rome, Italy: Prof. Nunzio Del Gaudio (Associate Professor)

**Abstract**:
Background: Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor in adults, characterized by rapid proliferation, high intratumoral heterogeneity, and poor prognosis. Beyond genetic alterations, epigenetic dysregulation plays a central role in GBM pathogenesis, particularly through Polycomb repressive complexes PRC2 and PRC1, which coordinate transcriptional silencing via H3K27 methylation and chromatin compaction. Emerging evidence suggests that metabolic–epigenetic coupling influences chromatin regulation through S-adenosylmethionine (SAM) availability. METTL16, a non-canonical RNA methyltransferase, regulates MAT2A expression, the key enzyme for SAM biosynthesis, suggesting a role in epigenetic–transcriptional crosstalk in GBM. Methods: METTL16 expression was analyzed across TCGA, GTEx, and CPTAC datasets and validated in GBM cell lines (U87, T98G) and patient-derived glioma stem-like cells. GEPIA3 analysis assessed transcriptional correlations between METTL16 and epigenetic regulators. Functional studies were performed using shRNA-mediated knockdown and pharmacological inhibition. Cellular phenotypes were evaluated through proliferation, clonogenicity, metabolic activity, and apoptosis assays. Transcriptomic changes were analyzed by RNA sequencing followed by Gene Set Enrichment Analysis and validated by RT-qPCR and immunoblotting. Integration with MeRIP-seq enabled identification of METTL16-associated transcripts. Results: METTL16 was significantly overexpressed in GBM models. GEPIA3 analysis revealed positive correlations between METTL16, EZH2, and CBX2 expression. Consistently, RNA-seq following METTL16 inhibition showed strong downregulation of EZH2 and marked reduction of CBX2. Additional Polycomb components were affected, including significant decrease in SUZ12 and moderate but consistent reduction of RNF2, indicating coordinated disruption of PRC2-mediated H3K27 methylation and PRC1-dependent chromatin compaction. Functionally, METTL16 inhibition impaired proliferation, clonogenic capacity, and metabolic activity while inducing apoptosis. A concomitant decrease in MAT2A expression was also observed, suggesting a link between METTL16 activity, SAM metabolism, and epigenetic regulation. Conclusions: These findings identify METTL16 as a key regulator of GBM cell survival and highlight its role in maintaining Polycomb-mediated epigenetic repression. The concordance between GEPIA3 correlations and RNA-seq validation supports a functional association between METTL16 and PRC components. The coordinated downregulation of EZH2, SUZ12, CBX2, and RNF2 suggests destabilization of chromatin silencing networks, potentially mediated by epigenetic–metabolic crosstalk. Targeting METTL16 may represent a promising therapeutic strategy in GBM.

↑ Beginning

# Exploring the epigenetic mechanisms underlying the evolution of CRC drug-tolerant persister cells

Only Poster | 1. Clinical cancer epigenetics

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- 2) IFOM ETS, The AIRC Institute of Molecular Oncology, Milan, Italy: Dr. Francesco Ferrari (Group Leader)

**Abstract**:
Background: Despite targeted therapies revolutionized the treatment of colorectal cancer (CRC), a subpopulation of drug-tolerant persister cells (DTPs) evades the lethal pressure of therapy through non-genetic mechanisms, thereby constituting a reservoir from which heterogeneous mechanisms of resistant eventually emerge, ultimately leading to tumor relapse and treatment failure. DTPs exhibit remarkable phenotypic plasticity and evolvability, enabling survival under drug pressure and reversion to a drug-sensitive state upon treatment withdrawal. Elucidating non-genetic regulatory mechanisms of DTPs in CRC is critical to understand tumor recurrence and uncover novel therapeutic vulnerabilities to prevent resistance and improve long-term treatment efficacy. Methods: CRC cells with different genetic-driven sensitivities to targeted therapies were treated with their specific regimens for at least 2 weeks to induce DTPs. After treatment, some DTPs were released from drug pressure until they regained sensitivity (DTPs-derived released), while others were continuously treated until resistance emerged (DTPs-derived resistant). Parental cells were used as control. Genome-wide approaches, including ATAC-seq and mass spectrometry of histone post-translational modifications (PTMs) were combined with pharmacological screenings of epigenetic remodelers inhibitors. Results: Preliminary data suggest a repressive chromatin accessibility profile in DTPs and a similarity with the resistant phenotype in terms of chromatin accessibility. Moreover, 63% of the analyzed H3 and H4 PTMs are significantly altered in DTPs compared to parental cells. From the pharmacological screening we found that HDAC and KMT1A inhibition with targeted therapy decreased residual DTPs viability in different CRC models. Further analyses are needed to confirm the involvement of these chromatin remodelers in DTPs phenotype. Conclusions: CRC DTPs display a repressive chromatin state with extensive histone PTMs alterations, suggesting an early adaptive phase toward resistance. Targeting epigenetic regulators such as HDAC and KMT1A was associated with reduced DTP viability, highlighting chromatin remodelers as promising vulnerability. Funding: This project is funded by My First AIRC Grant 26439- PI Mariangela Russo.

↑ Beginning

# Functional characterization of CBX2 inhibition in Glioblastoma

Only Poster | 1. Clinical cancer epigenetics

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- Department of Life Sciences, Health and Health Professions, Link Campus University, Via del Casale Di San Pio V 44, Rome, Italy: Prof. Nunzio Del Gaudio (Tenure Track Assistant Professor)

**Abstract**:
Background Polycomb group (PcG) proteins regulate epigenetic silencing and play key roles in development and lineage specification. Dysregulation of PcG components, including members of the Chromobox (CBX) family, is increasingly linked to oncogenesis. CBX2, the canonical chromatin reader of PRC1, has recently emerged as a potential oncogenic driver. Preliminary silico analyses indicate that CBX2 is markedly upregulated in glioblastoma (GBM), the most aggressive primary brain tumor in adults. Objectives This study aims to functionally characterize CBX2 in GBM by integrating reverse-genetic perturbations, phenotypic assays, and multi-omic profiling to elucidate its biological role and assess its potential as a therapeutic target. Methodology Differential expression analyses using TCGA and CGGA datasets revealed consistent upregulation of CBX2 in GBM relative to non-tumor controls. DepMap expression profiling was subsequently used to identify the most appropriate cellular models for functional studies. CBX2 expression levels were validated by qPCR and Western blot in SVGp12, U87, U138, and T98G cells, confirming low expression in U87, intermediate expression in U138, and high expression in T98G. Based on these profiles, U87 cells were selected for CBX2 overexpression using a GFP-tagged recombinant construct, whereas T98G cells were chosen for CBX2 knockdown via lentiviral shRNA vectors. Perturbation efficiencies were verified by qPCR and Western blot. The engineered cell lines were subsequently characterized through morphological assessment, viability, proliferation assays and colony formation assays. Results CBX2 knockdown in T98G cells induced marked morphological stress, followed by a rapid decline in viability and proliferative capacity. CCK-8 assays showed a >50% reduction in proliferation at all measured time points, accompanied by a loss of clonogenic potential. In contrast, CBX2 overexpression in U87 cells impaired both proliferation and colony formation, and unexpectedly also increased endogenous CBX2 expression, suggesting the presence of a potential positive autoregulatory feedback loop. Conclusions Collectively, these findings support a pro-oncogenic role for CBX2 in GBM, with its modulation exerting robust and directionally consistent effects on tumor cell growth. Additional molecular and multi-omic analyses are underway to delineate the pathways governed by CBX2 activity

↑ Beginning

# Investigating the effect of decitabine on the transcriptome of breast cancer cell lines.

Only Poster | 1. Clinical cancer epigenetics

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- University of Jeddah: Prof. Firoz Ahmed (Assistant Professor)

**Abstract**:
Introduction: Age is a significant risk factor in breast cancer, with younger age at presentation among Saudi patients compared to Western countries and premenopausal onset within (TNBC) subtype. Dysregulation of the neuroendocrine axis has been associated with both aging and cancer progression through its impact on immune responses and modifications of the TME. Both aging and cancer are marked by global DNA hypomethylation. Methods: This study examined how decitabine affects gene expression in two breast cancer cell lines T47-D (luminal A) and JIMT-1 (HER2-enriched). Data Acquisition: Buocikova et al. (2020) and a 2022 Frontiers in Pharmacology study. DEG: limma R package Enrichment Analysis: (GO) and KEGG pathway analysis via clusterProfiler. Horvath's epigenetic clock :aging-related hypermethylated genes. Survival: outcomes were assessed via Metabric dataset through cBioPortal, employing Kaplan-Meier plots and log-rank tests. Tumor Stage: correlated gene expression levels with tumor stage according to the Nottingham Prognostic Index. Results: In T47-D cells (Table1), decitabine led to 1,937 hypomethylated CpG sites and upregulated 248 genes, with some genes both hypermethylated and upregulated (Figure1). Pathway analysis identified cytoskeleton dynamics (KEGG) and cell-substrate adhesion (GO). KRT20 was notably hypermethylated and overexpressed, inversely correlating with age. Survival analysis found 114 associated genes, including CALML3, KRT5, CAV1, and ABCB1. In late-stage tumors, 13 genes were implicated, especially LRP8 (Figure4). For JIMT-1 cells, decitabine induced 1,195 hypomethylated CpGs and 187 upregulated genes, with a clear link between hypomethylation and gene upregulation (Figure2). KEGG highlighted neural signaling; GO featured ion transport and signaling. TFAP2E was upregulated and hypomethylated, positively correlated with aging. Eight survival-associated genes included F2RL1, GNG2, APOBEC3G, and HLA-G, with GNG2 most upregulated in late-stage tumors (Figure5). Both cell lines exhibited shared pathways involving piRNA-mediated epigenetic silencing (Figure3). Conclusion: DAC selectively alters age-associated CpGs, indicating targeted disruption of regulatory aging programs like oncofetal reprogramming, rather than overall epigenetic rejuvenation. This reinforces the need for subtype-aware evaluation, functional validation and biomarker-guided translation of epigenetic therapies in breast cancer. Funding: The authors thank Alfaisal University for institutional support.

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# Melanomas with mutations in chromatin-regulating genes are positively associated with mutational burden and improved response to checkpoint immunotherapy.

Only Poster | 1. Clinical cancer epigenetics

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- Aristotle University of Thessaloniki, Thessaloniki, Greece: Prof. Aimilios Lallas

**Abstract**:
Introduction: Melanoma is characterized by a high mutational load, largely driven by ultraviolet-induced DNA damage. Tumor mutational burden (TMB) has emerged as a predictive biomarker for response to immune checkpoint inhibitors (ICIs), yet the genomic determinants of TMB variability remain incompletely defined. Beyond canonical DNA repair defects such as mismatch repair (MMR) or POLE/POLD1 mutations, alterations in chromatin regulators (CRs) may reshape chromatin-templated processes and influence genome stability. Materials and Methods: We analyzed melanoma cohorts from three independent datasets, such as TCGA, MSK-Met, and MSK-ICI. TMB was compared between CR-mutant, CR–wild-type, and overall cohorts along with granular gene-level analyses, which evaluated individual CRs. Co-occurrence patterns were assessed by oncoprint analysis, linear regression, and Pearson correlation between the number of CR mutations and TMB. Overall survival (OS) in ICI-treated patients was evaluated using Kaplan–Meier curves and Cox proportional hazards models, with TCGA serving as a non–ICI-treated comparator. Results: Across all three datasets, melanomas harboring mutations in CR genes demonstrated significantly higher TMB compared with CR–wild-type tumors. At the gene level, several CRs, including ARID1A, DNMT3A, EP300, CREBBP, TET2, and NSD2, were associated with elevated median TMB, in some instances comparable to tumors with MMR or POLE/POLD1 mutations. Tumors carrying multiple CR alterations showed a strong positive linear relationship between the number of CR mutations and TMB. In the MSK-ICI cohort, CR-mutant tumors demonstrated significantly prolonged OS compared with CR–wild-type cases. Enrichment analyses further demonstrated that high-TMB CR genes were overrepresented among long-term survivors in the MSK-ICI dataset but not in non–ICI-treated cohorts. Conclusion: Mutations in chromatin regulator genes define a subset of melanomas with elevated tumor mutational burden and improved outcomes following immune checkpoint inhibition. The relationship between CR mutations and TMB appears partially independent of canonical DNA repair defects. Prospective validation is warranted to determine whether composite CR mutation signatures enhance the prediction of immunotherapy response in melanoma.

↑ Beginning

# Development of the tool for identification of phase specific epigenetic maintenance profile in healthy and tumor cells at single-cell resolution

Only Poster | 2. Epigenetic technologies

**Main author**: Sra. Sandra Binias (Sequencing Laboratory, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland · Bioinformatician)

**Co-authors**:

- Sequencing Laboratory, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland: Dr. Bartosz Wojtas (Head of the Lab)

**Abstract**:
Background Faithful maintenance of epigenetic states during cell division is essential for preserving cellular identity and genome function. In rapidly proliferating cells, this requires coordinated regulation of DNA methylation, histone modification, and chromatin organization across distinct phases of the cell cycle. In disease contexts, these mechanisms may become deregulated; however, their interpretation is complicated by the fact that many epigenetic regulators are inherently cell cycle dependent. Distinguishing pathological dysregulation from normal phase specific transcriptional dynamics remains particularly challenging in heterogeneous tissues. To address this, we performed a cell cycle aware analysis of single-cell RNA-seq data, aiming to compare healthy and tumor cells within matched proliferative states and to explore potential alterations in epigenetic maintenance programs. Results We analyzed two public colorectal cancer single-cell RNA-seq datasets from GEO (GSE161277 and GSE132465), comprising both healthy and tumor-derived cells. Using five complementary cell cycle scoring approaches, cells were assigned to G1, S, and G2/M phases, enabling phase-resolved comparisons between healthy and malignant compartments. Across datasets, we identified phase specific gene expression patterns, performed tumor vs healthy differential expression analyses within matched cell cycle states, and reconstructed candidate phase markers. We also assessed the consistency of these signals across datasets and phases. In addition, we compared phase resolved transcriptional signatures with a curated set of epigenetic regulators, allowing us to distinguish genes showing expected cell cycle dependent behavior from those potentially altered in tumor cells. These analyses demonstrate that a cell cycle aware framework can reveal structured transcriptional differences that would otherwise be confounded by proliferative state. Conclusions Our analysis provides a framework for studying transcriptional programs associated with epigenetic maintenance in a cell cycle aware manner at single-cell resolution. By integrating multiple phase assignment strategies with within phase comparisons across independent datasets, this approach enables identification of candidate regulators that are conserved, phase specific, or selectively altered in malignant cells. While still exploratory, this strategy establishes a basis for distinguishing general cell cycle effects from tumor associated dysregulation and can be further developed toward more formalized analytical pipelines and downstream applications.

↑ Beginning

# High-Throughput, Multiplexed, Quantitative Epigenetic Profiling of Cells, Tissues, and Liquid Biopsies

Only Poster | 2. Epigenetic technologies

**Main author**: Dr. Banushee Kumar (Epigenica AB · CTO)

**Co-authors**:

- Epigenica AB: Dr. Alisa Alekseenko (R&D Scientist); Dr. Carmen Navarro (Bioinformatician); Dr. Simon Elsässer (Co-founder)

**Abstract**:
Epigenetic changes drive different disease progression and can be detected early in the presymptomatic phases. Combined histone posttranslational modifications (hPTMs) and DNA methylation analyses reveal tumor origins and subtypes, help stratify patients, and identify epigenetic alterations in gene regulatory elements that fuel therapy resistance—yet existing methods fall short on throughput and quantitative precision for scalable insights EpiFinder™ GenomePro delivers multiplexed ChIP-seq for up to 8 hPTMs across 24 samples in one streamlined workflow. Its pool-split design slashes technical noise while enabling spike-in-free, quantitative comparisons between conditions. Compatible with native/formalin-fixed cells and frozen tissues, it integrates DNA methylation profiling for holistic multi-layer epigenomic analysis. EpiFinder™ cNUC pioneers high-throughput, multiplexed, quantitative epigenomics from plasma/serum nucleosomes—no extraction needed. This first-of-its-kind platform profiles hPTMs and DNA methylation simultaneously across liquid biopsies, unlocking novel biomarker signatures for cancer monitoring. EpiFinder's high-throughput, multiplexed, quantitative platforms revolutionize epigenetic profiling of cancer cells, tissues, and liquid biopsies—accelerating diagnostics, precision therapies, and drug discovery.

↑ Beginning

# The 6-base genome unlocks novel mechanistic biomarkers and enhanced biological insight from challenging clinical samples

Only Poster | 2. Epigenetic technologies

**Main author**: Sascha Seidel (biomodal)

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**Abstract**:
Background DNA methylation is now understood to function as both an activating and deactivating regulatory signal. However, most epigenetic sequencing technologies obscure this complexity by collapsing distinct cytosine modifications. Current methods either report a composite modC signal that conflates activation and repression or measure 5-methylcytosine (5mC) alone, capturing only deactivating information. These limitations restrict biological interpretation, reduce confidence in results, and, for example, lead to missed differentially methylated regions (DMRs) and misclassified regulatory element status. Methods We evaluated the impact of resolving individual cytosine modifications using duet evoC, a 6-base sequencing approach that simultaneously measures 5mC and 5-hydroxymethylcytosine (5hmC) with market-leading accuracy. Performance was assessed across diverse datasets and sample types, including challenging clinical materials such as FFPE tissue and cfDNA. Analyses compared insights derived from 6-base data against those obtainable from composite modC and 5mC-only approaches. Results Separating activating (5hmC) and deactivating (5mC) signals revealed biologically meaningful DMRs that were missed by modC methods and corrected erroneous interpretations derived from 5mC-only data. These differences were particularly pronounced in enhancer regions, where activating and repressive marks coexist and dynamically regulate gene expression. Six-base resolution enabled more accurate enhancer state annotation, high performance inference of gene expression, and early detection of activating biomarkers. In addition, integrating 5mC and 5hmC into 6-base end motifs and combining with genetic information generated more powerful multiomic biomarkers than genetic or epigenetic data alone. Conclusions duet evoC enables complete epigenetic insight by delivering accurate, robust 6-base data from both high-quality and challenging clinical samples. As a fully integrated solution encompassing assay, data processing, and analysis in a single workflow, duet evoC provides comprehensive epigenetic context, allowing researchers to ask the right biological questions—and answer them with confidence.

↑ Beginning

# TIP-ChIP: Tagmented, Indexed, and Pooled ChIP-Seq

Only Poster | 2. Epigenetic technologies

**Main author**: Dr. Anne-Sophie Berthomieu (Active Motif SA, Waterloo, Belgium · Field Application Scientist)

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**Abstract**:
TIP-ChIP, a high-throughput chromatin profiling assay, offers significant advantages over traditional ChIP-Seq. Results demonstrate substantial reductions in hands-on time while reducing sample-to-sample variance and improving reproducibility across experiments. Histone marks and transcription factors were profiled across multiple samples, and the Tn5-based barcoding strategy enabled accurate deconvolution of individual samples without loss of resolution. In primary neutrophils treated with PMA, TIP-ChIP detected distinct H3K27ac changes, highlighting dynamic chromatin responses to stimulation. In LPS-treated THP-1 cells, TIP-ChIP captured time-resolved shifts in H3K27ac, H3K4me3, RNA polymerase II Ser2P, and NF-κB (p50), revealing coordinated enhancer, promoter, and transcriptional changes during the inflammatory response.

↑ Beginning

# Variable gene regulatory responses to epigenetic editing across distinct epigenetic chromatin contexts

Only Poster | 2. Epigenetic technologies

**Main author**: Sra. Anna van den Berg van Saparoea (Molecular and Cellular Epigenetics Group, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands · PhD Candidate)

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- Epigenetic Editing Research Group, Department of Pathology and Medical Biology, University Medical Center Groningen, The Netherlands: Prof. Marianne Rots (Professor)
- Department of Medical Biochemistry, Amsterdam UMC, University Medical Centers, The Netherlands: Prof. Pernette Verschure (Professor)

**Abstract**:
To fully exploit the potential of CRISPR/deactivatedCas9-based systems for epigenetic editing, especially in a sustained and controllable manner, it is essential to optimize and validate the performance of generated epigenetic editors prior to applying them in CRISPR-perturbation screens. As the epigenetic context in which a gene is situated has a major effect on its transcriptional activity and heterogeneity across cells, evaluating each set of epigenetic editors within the appropriate genomic and epigenetic chromatin context is crucial, yet remains challenging. We generated a multitude of epigenetic editor constructs by fusing diverse enzymatic domains to dCas9 and blue fluorescent protein. To evaluate their activity, we established multiple reporter cell lines targeting either endogenous or engineered loci with defined epigenetic contexts, using single or multiple guide RNAs. The epigenetic context of target genes was defined using a chromatin state model generated from public epigenomic datasets from the appropriate cell lines. Using the reporter cell lines, our epigenetic editor constructs were tested in a transient manner on short and longer-term effects. We used RT-qPCR for bulk RNA measurements and flow cytometry for single-cell analysis on protein level to read-out the effects of our designed epigenetic editor constructs. Preliminary results show large variability in the impact of epigenetic editing on gene expression, not just between effects of different editors and target genes within their specific epigenetic contexts, but also between biological replicates. Whilst DNA methylation in combination with KRAB-domain induced silencing results in a robust and sustained manner in several epigenetic contexts, the inverse is not consistently observed when attempting to (re-)upregulate the same reporter gene using a DNA demethylase. In addition, we noted H3K4me3 induced gene activation combined with H3K27me3 erasure-based de-repression results in levels of target gene upregulation that are highly variable between replicates. Our findings highlight the importance of taking concepts such as transcriptional heterogeneity, chromatin state, and the bivalence of target genes into account when interpreting the regulatory effects of epigenetic editors.

↑ Beginning

# CBX6 as an epigenetic regulator of differentiation in Glioblastoma

Only Poster | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by pronounced epigenetic plasticity and maintenance of a stem-like, therapy-resistant state. Chromebox 6 (CBX6), a component of the canonical PRC1 complex, is consistently downregulated in GBM. Its reduced expression correlates with higher tumor grade and IDH wild-type status, suggesting a potential tumor-suppressive role. However, the functional contribution of CBX6 to GBM biology remains poorly understood. Methods: CBX6 expression was analyzed in silico (GEPIA2, CGGA, TCGA) and validated by RT-qPCR, WB, and IF in GBM vs normal glial cells. CBX6 OE U87-MG cells were generated by lentiviral transduction. Functional assays included CCK-8, FACS, scratch, TMZ (temozolomide) sensitivity, and colony formation. ATRA-induced differentiation (5 μM) was assessed via NES, MAPT, and β-Tubulin III by RT-qPCR, WB, and IF. Results CBX6 expression was significantly reduced in GBM compared with normal brain tissue, with the lowest levels observed in WHO grade IV, IDH wild-type tumors. In vitro validation confirmed CBX6 downregulation at both mRNA and protein levels in GBM cell lines. To investigate its functional role, a stable CBX6-OE GBM cell model (U87-MG) was generated. CBX6 overexpression did not significantly affect cell viability, cell cycle progression, migration, or temozolomide sensitivity; however, it markedly impaired clonogenic capacity, suggesting a role in modulating tumor cell identity. ATRA-induced differentiation in U87 cells resulted in a progressive increase in CBX6 expression, accompanied by downregulation of the stemness marker Nestin and upregulation of the neuronal marker MAPT. Consistently, preliminary immunofluorescence analyses in the CBX6-OE GBM cell model indicated reduced Nestin levels and increased β-Tubulin III expression, further supporting a role for CBX6 in promoting differentiation. Conclusions These findings identify CBX6 as a potential epigenetic regulator of cancer cell identity in GBM, whose loss may contribute to the maintenance of stem-like features and increased tumor aggressiveness. Ongoing studies aim to further elucidate the CBX6-dependent transcriptional and chromatin programs using RNA-seq and ATAC-seq approaches, to evaluate its role in promoting differentiation across multiple GBM models.

↑ Beginning

# DNA Methylation Reconfiguration in Temozolomide-Resistant Recurrent Glioblastoma: a Comparison between epigenetic dynamics in vitro and in vivo

Only Poster | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Glioblastoma is the most aggressive form of brain tumor and it is associated with poor clinical outcomes. The current standard of care includes surgical resection followed by radiotherapy and a Temozolomide (TMZ)-based chemotherapy. However, the effectiveness of TMZ is limited by its toxicity and the emergence of drug resistance. The methylation status of the MGMT promoter is a key prognostic and predictive biomarker of TMZ response, and loss of MGMT promoter methylation is a major driver of chemoresistance. However, drug resistance is not only due to changes in MGMT methylation. The entire epigenome changes and acquires specific features which leads to tumor progression. Results To investigate epigenetic features associated with TMZ resistance, we developed a TMZ-resistant cell model and analyzed the epigenetic remodeling following prolonged drug exposure in cells. These findings were compared with genome-wide DNA methylation profiles from a cohort of patients with recurrent glioblastoma. We first characterized the epigenetic alterations induced by TMZ in vitro, then we examined the differences between primary and recurrent tumors in patient samples. At the end, we performed an integrative analysis to identify shared epigenetic signatures between TMZ-resistant cells and recurrent glioblastomas, with the aim of uncovering features of resistance and potential therapeutic targets to explore in the future. Our data indicate that TMZ induces important epigenetic reprogramming in Glioblastoma, contributing to both treatment resistance and increased tumor aggressiveness. Conclusions These results highlight the importance of further investigating DNA methylation dynamics in TMZ resistance. Further studies using primary tumor-derived cells may help to identify strategies to overcome chemoresistance.

↑ Beginning

# Epigenetic Editing–Mediated Regulation of PPARGC1A to reprogram Mitochondrial Function and Suppress Colon Cancer Progression

Only Poster | 3. Epigenetic mechanisms in human disease

**Main author**: Sr. Yiheng Shi (Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen · Phd candidate)

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**Abstract**:
Mitochondrial dysfunction contributes to metabolic reprogramming and tumor progression in colon adenocarcinoma (COAD). We aimed to identify key mitochondria-related regulators in COAD to be reprogrammed by epigenetic editing1 to modulate mitochondrial function and suppress tumor progression. Transcriptomic and clinical COAD data were obtained from TCGA (n=514) and validated in GSE39582 (n=585) datasets. Diagnostic/prognostic values were evaluated by Receiver Operating Characteristic Curve, Kaplan-Meier and Cox regression analyses. A prognostic model was constructed and evaluated. Associations between gene expression and clinical features were analyzed using correlation analysis, while functional pathways were explored using gene set enrichment analysis. CRISPR-dCas9 activation (VPR, VPR+TET, PRDM9+TET, p300+TET) or repression systems (CRISPROFF=KRAB&Dnmt3A3L, SKD+DNMT3B) were transiently expressed with guideRNAs in non-cancer (HEK293T, LLC-PK1) and colon cancer (HCT116) cells, expression modulation was assessed by RT-qPCR. Functional effects were assessed using MitoSOX and hypoxia response element (HRE) luciferase assays. PPARGC1A expression was downregulated in COAD compared with normal colon and low expression associated with advanced tumor stage, microsatellite instability and worse overall survival (HR:0.581, p=0.012). ROC analysis showed good diagnostic performance (AUC=0.925) and PPARGC1A was identified as an independent prognostic factor (p=0.023). The prognostic model demonstrated good predictive accuracy with AUCs of 0.756 (95%CI:0.675–0.836), 0.767 (95%CI:0.695–0.839), and 0.743 (95%CI:0.644–0.841) for 1-, 3-, and 5-year survival, respectively. Functional enrichment analysis confirmed that PPARGC1A coexpressed genes were mainly enriched in mitochondrial and metabolic pathways. In HEK293T, PPARGC1A could be upregulated, while CRISPR-repression systems had limited impact. In LLC-PK1 cells, PPARGC1A activation did not consistently alter ROS levels. CRISPRoff-mediated repression increased cellular response to hypoxia. In HCT116, VPR induced upregulation, and epigenetic editors including PRDM9+TET also significantly enhanced PPARGC1A expression 5 days post-transfection. Next, we will investigate whether targeted modulation of PPARGC1A alters mitochondrial copy number, transcriptional activity, and key functional outputs in COAD. Long-term epigenetic reprogramming of PPARGC1A and/or related genes may provide a novel strategy to modulate mitochondrial function and to inhibit tumor growth.

↑ Beginning

# Epigenomic signatures and accelerated epigenetic aging in major depressive disorder: preliminary results from the OPADE project

Only Poster | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Major depressive disorder (MDD) is one of the most prevalent and disabling psychiatric conditions worldwide, affecting approximately 280 million individuals. Despite the availability of multiple pharmacological treatments, clinical outcomes remain largely unsatisfactory, with low remission rates and a substantial proportion of patients showing limited or no response to therapy. This heterogeneity underscores the urgent need for objective molecular biomarkers capable of predicting treatment response and supporting personalized therapeutic strategies. The OPADE project (EU Horizon Europe, GA No. 101095436) is an international multicenter initiative involving 14 research and clinical centers across 10 countries, aiming to integrate multi-omics data to identify clinically relevant molecular signatures in MDD. Within this framework, CEINGE is responsible for genomic and epigenomic profiling of patients longitudinally followed during pharmacological treatment. Results Preliminary analyses have been conducted on an initial cohort of approximately 100 patients, for whom genome-wide DNA methylation profiles were generated at baseline and after one year of treatment. Early findings indicate a statistically significant increase in epigenetic age compared to chronological age across samples, suggesting a pattern of accelerated epigenetic aging in patients with MDD. Furthermore, longitudinal comparisons between baseline and follow-up samples reveal more than 100,000 differentially methylated sites, indicating extensive and dynamic epigenomic remodeling associated with disease state and/or treatment exposure. Ongoing analyses are focusing on characterizing inter-individual epigenetic distances and the temporal dynamics of methylation changes during therapy. Conclusions These preliminary results suggest that major depressive disorder is associated with widespread epigenomic alterations and a measurable acceleration of epigenetic aging. The large number of differentially methylated sites observed over time highlights the dynamic nature of the epigenome in this clinical context. Integration of these data with metabolomic, proteomic, and microbiome profiles within the OPADE consortium will contribute to the identification of robust molecular signatures of disease and treatment response, ultimately supporting the development of precision medicine approaches in psychiatry.

↑ Beginning

# Exploring the therapeutic potential of Histone Deacetylase 11 (HDAC11) in Diabetic Cardiomyopathy and Heart Failure

Only Poster | 3. Epigenetic mechanisms in human disease

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**Abstract**:
BACKGROUND: Diabetic cardiomyopathy (DCM) is a major cause of heart failure and mortality in diabetes, independently of other cardiovascular risk factors. Epigenetic mechanisms contribute to DCM and heart failure by regulating gene expression, metabolic pathways, and cellular stress responses. This study investigated novel epigenetic mechanisms involved in diabetic cardiac injury, focusing on histone deacetylase 11 (HDAC11), its transcript isoforms, and potential regulation by microRNAs and N6-methyladenosine (m6A) modification. METHODS: Computational analyses of HDAC family members and m6A regulators were performed using RNA-seq data from high-glucose–treated AC-16 cardiomyocytes, integrated with GEPIA/GTEx, m6A-Atlas, and UCSC Genome Browser resources to evaluate HDAC11-associated m6A regulation and transcript isoforms. An in vitro model of diabetic cardiomyopathy and heart failure–like injury was established in AC-16 cells exposed to high glucose (25mM) for 48 h, followed by doxorubicin for 24 h at the experimentally determined IC50. HDAC11 expression/localization and PBMC-derived intracellular/circulating miRNA profiles were assessed in clinically stratified cohorts. RESULTS: High-glucose exposure significantly upregulated HDAC11 in AC-16 cardiomyocytes, with an early increase (2-16 h) followed by a later decline, suggesting involvement in the acute metabolic stress response. Under diabetic cardiotoxic conditions, high glucose plus doxorubicin selectively increased the canonical HDAC11 isoform, whereas alternative isoform 3 was reduced, indicating isoform-specific regulation during heart failure–like injury. HDAC11 appeared predominantly cytosolic under basal conditions, while cardiotoxic stress suggested possible nuclear relocalization and transcriptional reprogramming. Intracellular miRNA profiling identified miR-145-5p as a putative upstream regulator of HDAC11. CONCLUSION: These findings identify HDAC11 as an early epigenetic responder to metabolic and cardiotoxic stress. Its isoform-specific regulation, stress-dependent relocalization, and possible modulation by miR-145-5p may define an epigenetic–post-transcriptional axis in diabetic heart failure, suggesting HDAC11 as a potential biomarker and therapeutic target. Future in vivo validation in sham, transverse aortic constriction, and doxorubicin-induced cardiotoxicity mouse models, potentially combined with diabetic/metabolic stress, will clarify HDAC11 involvement in cardiac remodeling, cardiotoxic injury, and heart failure.

↑ Beginning

# HDAC7 restrains Diffuse Large B Cell Lymphoma progression and enhances immunochemotherapy response through upregulation of CD20

Only Poster | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Diffuse Large B-Cell Lymphoma (DLBCL) is the most common aggressive B-cell malignancy, and despite R-CHOP remaining as the standard-of-care therapy, approximately 40% of patients relapse or are refractory to treatment. Loss of CD20 surface expression is an established mechanism of rituximab resistance and, consequently, one of the major causes of relapse. However, the epigenetic regulators guiding CD20 (encoded by MS4A1 gene) transcription remain poorly understood. HDAC7, a class IIa histone deacetylase with reported tumor suppressor functions in B-cell malignancies and associated with an improved outcome, emerges as a candidate regulator of this axis. Methods HDAC7 and CD20 expression were analyzed in DLBCL patient cohorts and cell lines. A doxycycline-inducible HDAC7 overexpression system was generated in HDAC7-deficient DLBCL cell lines (MD-901, K-231), while loss-of-function was achieved via shRNA knockdown in high-HDAC7 cells (VAL, RIVA). Treatment response was assessed using Annexin V/7-AAD apoptosis assays and 3D spheroid models. Mechanistic studies include RNA sequencing and chromatin immunoprecipitation quantitative PCR (ChIP-qPCR) profiling H3K27ac and H3K27me3 histone marks at MS4A1 promoter. For in vivo validation, subcutaneous xenograft model was conducted in immunodeficient mice, using HDAC7-overexpressing K-231 cells. Results High HDAC7 expression associated with improved overall survival in DLBCL patients and positively correlated with CD20 expression. HDAC7 induction in deficient cell lines consistently upregulated CD20 at mRNA and protein levels while conferring higher sensitivity to R-CHOP in both cultured and spheroid cells. Conversely, HDAC7 knockdown reversed this differential response to rituximab. Mechanistically, ChIP-qPCR data revealed that HDAC7 promotes an active chromatin state at the MS4A1 promoter, evidenced by H3K27ac enrichment and depletion of H3K27me3. Whole transcriptomic profiling revealed that HDAC7 induction promotes B cell maturation and enhances tumor suppression. Finally, our xenograft model demonstrated that, HDAC7 suppressed tumor growth and dramatically enhanced efficacy of Rituximab treatment as single agent, achieving almost complete tumor regression. Conclusions These findings establish HDAC7 as a regulator of CD20 transcription and a determinant of immunochemotherapy response in DLBCL. Therefore, we propose that HDAC7 induction is a potential therapeutic strategy to overcome rituximab resistance. Funding This work was supported by grants PI21/01451, PID2024-160270OB-I00 and DJCLS/07R/2022.

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# Insights into the biological role of LAV-BPIFB4: epigenetic mechanisms and possible therapeutic implications

Only Poster | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Epigenetics studies the regulation of gene expression that occurs without changes in the DNA sequence (Al Aboud et al., 2024). To date, a variant of the BPIFB4 gene associated with longevity (LAV-BPIFB4) has been identified but little is known about its implication in pathophysiological processes as cell alterations, aging, metabolism (Ciaglia et al., 2025). LAV-BPIFB4, most commonly found in centenarians (Villa et al., 2015), appears to play a protective role in cardiovascular and aging-associated processes by shifting the balance from pathological aging toward healthy aging (Cattaneo et al., 2023). In this study, we focused on analyzing the epigenetic mechanisms underlying the regulation of LAV-BPIFB4 and how these processes can be utilized to explore its involvment in biological mechanisms underlying healthy aging. Methods The data were organized based on the identification of stratification criteria such as genotype status, the presence of known resistance and their potential coexistence. The effect of resistant and sensitive patient serum was analyzed by using sequencing techniques. The metabolic activity on HUVEC cells was evaluated using Seahorse analysis while protein modifications associated with epigenetic patterns were analyzed by Western blot. Finally, levels of lactate, LDH and α-ketoglutarate were quantified using ELISA assays. Results LAV-BPIFB4 is able to induce a differential response between sensitive and resistant samples. We found a reduction of basal respiration in sensitive samples while an increase in resistant samples with modulation of the proton leak. Transcriptomic analysis identified genes (DGE) and pathways involved in thrombosis, DNA repair, inflammation. In resistant phenotypes, a modulation in acetylation/lactylation markers is also observed, while ketoglutarate metabolism seems to show a genotype dependent effect. Conclusions The results indicate that LAV-BPIFB4 plays a significant role in metabolism, epigenetic status, transcriptional programs. These findings suggest potential new biomarkers and therapeutic targets but further studies are needed to clarify its role during aging. Funding This work was funded by PNRR-MAD-2022-12376723 The longevity-associated variant of BPIFB4: a novel tool against thrombocytosis and “aspirin resistance” in diabetes CUP: B83C22006740006; EPI-MET-Funzionalizzazione delle aberrazioni (epi)genomiche nei tumori metastatici - Prog n. F/310034/03/X56 CUP: B29J24000550005.

↑ Beginning

# LINC complex disruption impairs DNMT1 dynamics and nuclear lamina organization in RPE-1 cells

Only Poster | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Tumor development is frequently associated with profound alterations in nuclear architecture, often caused by alterations in the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex and loss of the proper maintenance of DNA methylation patterns. The LINC complex ensures the connection between the cytoskeleton and the nucleoskeleton. Alterations in this complex are observed during physiological aging and in pathological conditions such as progeria, leading to disorganization of lamina-associated domains (LADs) and chromatin decompaction, which may drive senescence and neoplastic transformation. Global DNA hypomethylation is frequently observed in both tumor and aged cells, in the absence of mutations in genes encoding DNA methyltransferases (DNMTs), such as the maintenance enzyme DNMT1. This would suggest alternative mechanisms that impair its activity. In this context, we investigated whether LINC complex impairment has an impact on DNMT1 regulation. Methods RPE-1 cells engineered to express the endogenous DNMT1 fused to mNeonGreen were used to monitor DNMT1 dynamics by fluorescence microscopy. Disruption of the LINC complex was induced by cell transfection with a plasmid expressing a dominant-negative form of Nesprin-2 (DN-KASH), together with an mCherry reporter to distinguish transfected cells. DN-KASH-negative and -positive cells were then sorted by FACS. DNMT1 expression was also evaluated by Western blot and RT-PCR. Potential defects in nuclear lamina components were assessed by Western blot. Results Our analyses revealed that DNMT1 is strongly reduced only in DN-KASH-expressing cells. This reduction was not due to transcriptional changes; however, we observed recovery of DNMT1 upon cell treatment with a proteasome inhibitor, indicating post-translational degradation as the mechanism involved in the reduction. Interestingly, our data showed that DN-KASH-expressing cells also exhibit reduced Lamin B1, suggesting dysregulation of the nuclear lamina. Conclusions Overall, our data suggest that impairment of the nucleus–cytoskeleton connection may directly affect DNMT1 nuclear dynamics and nuclear lamina alterations, highlighting a potential link between nuclear mechanical alterations and epigenetic dysregulation in aging and tumorigenesis. This work was supported by EuroStart.

↑ Beginning

# HAT4 regulates muscle integrity and function through epigenetic modulation of MEF2C in zebrafish

Only Poster | 4. Epigenetic and chromatin in stemness, aging and development

**Main author**: Sra. Rabia Basri Javaid (Università degli Studi della Campania “Luigi Vanvitelli” · Phd Student)

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**Abstract**:
Epigenetic regulation is crucial for maintaining tissue homeostasis and coordinating gene expression during development and aging. Histone acetyltransferase 4 (HAT4) which is a Golgi-localized N-terminal acetyltransferase responsible for modifying membrane associated proteins and is involved in maintaining the structure of organelles. However, its developmental functions need to be understood. Preliminary evidence suggested that the loss of histone acetylation can disrupt transcriptional programs essential for muscle differentiation. Disruption of acetylation patterns has been found to be associated with congenital myopathies, impaired muscle regeneration, developmental deformities, and age associated decline. No previous studies have described how HAT4 regulates tissue architecture nor weather its loss leads to muscular deformities. In this study, we investigated the structural, molecular, and functional consequences of HAT4 loss using a zebrafish HAT4C-Δ126/183aa model. Muscle organization was first assessed by birefringence analysis, which revealed reduced light intensity with compromised muscle integrity in mutants. To further investigate the molecular basis of these abnormalities, we performed western blot analysis focusing on MEF2C which is a key transcription factor involved in muscle differentiation and maintenance. Mef2c protein levels in mutants are significantly reduced indicating a transcriptional link between HAT4 and MEF2C. Moreover, we performed the behavior experiments to assess the functional impact of molecular and structural changes. Anxiety like behavior with less exploration was observed in mutants which develop a link between epigenetic regulation and muscle structure. We further aim to characterize the age associated phenotypes in mutant model through β-galactosidase staining, telomerase aging and analysis of other muscle-specific markers. These investigations will assist in determining whether HAT4 plays a role in controlling muscle longevity and ageing. Our research concludes that HAT4 is a crucial regulator of muscle integrity and function, possibly via epigenetic modification of MEF2C. This work offers new insights into the chromatin-based regulation of muscle biology and highlights the significance of epigenetic variables in age-related functional deterioration.

↑ Beginning

# Identification of a putative cis-regulatory element of the human NANOG gene in cancer stem cells

Only Poster | 4. Epigenetic and chromatin in stemness, aging and development

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**Abstract**:
Background The transcription factor NANOG plays a central role in maintaining pluripotency and self-renewal in embryonic stem cells, and its aberrant overexpression has been widely described in multiple human cancers, including gliomas and embryonal carcinomas. Despite its conserved biological function, the regulatory mechanisms governing NANOG expression in human stem cells remain poorly understood, particularly regarding the contribution of cis-regulatory elements (CREs). While genome-wide studies have identified putative CREs, none have been definitively validated as essential regulators of NANOG in human cells, in contrast to well-characterized mouse models. Methods Building on previous findings about the epigenetic regulation of the mouse Nanog locus, this study aims to investigate similar regulatory mechanisms in human cancer stem cell models. Four human cell lines were selected: NCCIT (embryonal carcinoma), PA-1 (ovarian teratocarcinoma), and two glioma stem cell (GSC) lines, SD2 and SD3. Results Expression analysis confirmed elevated levels of stemness markers, including NANOG and SOX2, particularly in NCCIT and SD3 cells, while SD2 and SD3 also exhibited high expression of neural stem cell markers such as NESTIN and OLIG2. Bioinformatic analysis of the NANOG genomic region using the UCSC Genome Browser identified bidirectional transcription peaks approximately 3 kb upstream of the transcription start site (TSS), suggestive of enhancer RNA (eRNA) activity. Experimental validation through reverse transcription and walking PCR led to the identification of a transcript originating from this region. Preliminary experiments through CRISPR-Cas9 approach have been performed to delete the putative region of the eRNA. Conclusions Overall, this study provides preliminary evidence for the existence of a putative regulatory element upstream of the human NANOG gene. Future work will focus on functional characterization of this region to assess its impact on NANOG expression, stemness maintenance, and tumorigenic potential, thus contributing to a deeper understanding of NANOG regulation in human cancer.
Funding Thanks to CEINGE – Biotecnologie Avanzate Franco Salvatore (Napoli, Italy) and IJC – Josep Carreras Leukaemia Research Institute (Badalona, Barcelona, Spain) facilities. Thanks to Dr Roland Friedel who kindly donated GSCs.

↑ Beginning

# Identifying Age-Associated DNA Methylation Sites and Their Functional Relevance Using Epigenetic Editing in Dermal Fibroblasts

Only Poster | 4. Epigenetic and chromatin in stemness, aging and development

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**Abstract**:
Background: Dermal fibroblasts play an important role in maintaining skin structure and function. Although studies have described an imbalance of the proteins that promote and modulate structural integrity in aged dermal fibroblasts, a clear molecular mechanism has not been defined. Epigenetic modifications such as DNA methylation have been increasingly promoted as a robust marker for aging. Indeed, machine learning algorithms such as the skinHorvath clock can predict age of an individual using methylation data of specific CpG sites. However, the functional relevance of many such CpG sites remains to be understood. Methods: Aging-associated gene sets were curated from literature and their validity tested in two independent transcriptomic datasets using Gene Set Variation Analysis. DNA methylation profiles were analyzed using in-house (n=13) and publicly available (n=25) datasets. Aging-associated differentially methylated CpGs were identified and validated in two additional independent public datasets (n=52), followed by pyrosequencing in primary dermal fibroblasts. Deactivated Cas9-based epigenetic editing was performed to evaluate the functional consequences of induced methylation at these regions. Results: Among the curated aging-associated differentially expressed gene sets, only three gene sets showed a significant aging-associated trend and only in one transcriptomic dataset, confirming limited reproducibility of gene expression changes in cultured dermal fibroblasts. Contrastingly, DNA methylation analysis identified 3 CpGs that consistently showed statistically significant aging-associated DNA methylation. These three CpGs are also part of the skinHorvath clock. Importantly, 2 CpGs localized within one CpG island, annotated to a gene promoter, and differential methylation was confirmed by pyrosequencing in primary dermal fibroblasts. Epigenetic editing using CRISPROff induced methylation at the identified region in HEK293T cells, resulting in sustained gene repression (>29 days). These CRISPR tools will shed light on regulatory functions of age-associated candidate genes and their downstream targets. Conclusions: DNA methylation is a robust biomarker for aging in dermal fibroblasts. Next to providing research tools, this study highlights epigenetic editing as a potential strategy to rejuvenate old skin. Financed by EpiGuideEdit (project number KICH1.ST01.20.045) of the research program KIC Key Technologies 2020, which is partly financed by the Dutch Research Council (NWO).

↑ Beginning

# Defining novel therapies to prevent lineage switch in B lymphocyte malignancies through HDAC7 induction

Only Poster | 5. Epigenetics in drug discovery and therapeutics

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**Abstract**:
Background: Infant pro-B acute lymphoblastic leukemia (pro-B-ALL) with t(4;11) rearrangement presents an aggressive phenotype and very poor response to conventional chemotherapy. Moreover, immune escape mechanisms such as lineage switch (LS) involve the loss of B cell markers, leading to failure of immunotherapies against CD19. Our findings have demonstrated that histone deacetylase HDAC7, a key factor in B cell differentiation, plays an essential role in preventing the onset of pro-B-ALL in t(4;11) patients. In this sense, we have identified a novel precision therapy including Menin-1 inhibitors, that induces B cell differentiation through restoration of HDAC7 expression. Here we explore whether this therapeutic strategy could impair LS mechanisms, leading to a potential improvement of leukemic cells response to CD19 CAR-T therapy. Methods: In this project, in vitro experiments together with the use of primary leukemic cells as patient-derived xenografts (PDXs) and murine leukemia models were used, along with transcriptomics data, to decipher the mechanisms beyond HDAC7's role in LS. Results: First, after forced exogenous overexpression of HDAC7 in t(4;11) LS-AML cells, we found that it modifies the expression pattern of lymphoid/myeloid genes in vitro and in vivo. Moreover, RNAseq data shows that HDAC7 induction in LS-AML cells affects relevant processes such as transcription, RNA processing and chromatin remodeling, although it does not alter cell viability. After subjecting primary samples to cell sorting, we obtained PDXs enriched in leukemic cells with low levels of CD19 (CD19low), more prone to myeloid switching. Interestingly, when treating CD19low PDXs with different Menin-1 inhibitors, they displayed reduced cell viability than CD19high counterparts. Remarkably, this effect is dependent on Menin-1 inhibitors capacity to trigger HDAC7 expression. Conclusions: HDAC7 reverts LS in t(4;11) pro-B-ALL cells and LS-AML cells in vitro and in vivo. Interestingly, emerging epigenetic drugs such as Menin-1 inhibitors are more active against those cells with lower CD19 expression, through a specific induction of HDAC7. We propose HDAC7 induction as a promising therapeutic strategy to improve the response of t(4;11) pro-B-ALL cells that underwent relapse as AML to standard immunotherapies such as CD19 CAR-T. Funding: This work is supported by grants PI21/01451, PI25/00109, PID2024-160270OB-I00 and DJCLS/07R/2022.

↑ Beginning

# Epigenetically Driven Variability in Therapeutic Response in PDAC

Only Poster | 5. Epigenetics in drug discovery and therapeutics

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**Abstract**:
Abstract Background: Pancreatic ductal adenocarcinoma (PDAC) remains a highly lethal malignancy with limited treatment options. Epigenetic therapies targeting DNA methylation and chromatin regulation represent a promising avenue; however, their efficacy across different preclinical models remains insufficiently understood. Methods: We systematically evaluated the effects of a panel of epigenetic compounds representing distinct functional classes, including DNA methyltransferase inhibitors (Decitabine, Guadecitabine), bromodomain and extra-terminal domain (BET) inhibitors (JQ1, Apabetalone, ABBV-744), histone deacetylase (HDAC) inhibition (SAHA (Vorinostat), histone methylation modulators (Tazemetostat, Lirametostat), as well as additional epigenetically active compounds (XP-524, Curcumin), across a panel of 12 PDAC cell lines. Selected treatments and combinations, including gemcitabine-based regimens, were further assessed in patient-derived organoid models. Results: Across the cell line panel, responses to epigenetic therapies were largely homogeneous, suggesting consistent drug sensitivity under standard in vitro conditions. In contrast, organoid models displayed marked heterogeneity in treatment response, reflecting greater biological diversity and patient-specific variability. Among the tested strategies, XP-524 monotherapy and the combination of ABBV-744 with gemcitabine emerged as the most promising, demonstrating enhanced efficacy compared to single-agent treatments. Conclusions: These findings highlight a critical discrepancy between conventional cell line models and more physiologically relevant organoid systems. While cell lines suggest uniform sensitivity, organoids reveal substantial heterogeneity, underscoring the importance of model selection in preclinical drug evaluation. The observed benefit of combining BET inhibition with gemcitabine supports further investigation of such strategies in translational and clinical settings. This study was supported by APVV-21-0197, APVV-20-0143, TRANSCAN2023-1858-117, COST Action grants CA21135, CA21116, CA24162, APD0045 and APD0135.

↑ Beginning

# Small molecule inhibition of NUP98::KDM5A eliminates leukemic dependencies inducing differentiation in AML models

Only Poster | 5. Epigenetics in drug discovery and therapeutics

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**Abstract**:
N/A

↑ Beginning

# A plasma methylation model for tumor fraction estimation to deconfound epigenetic drivers of therapy resistance in metastatic colorectal cancer

Only Poster | 7. Epigenetic biomarkers

**Main author**: Sr. Daniel G. Camblor (INCLIVA Biomedical Research Institute, Valencia, Spain)

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**Abstract**:
Background Therapy resistance in metastatic colorectal cancer (mCRC) remains a major, poorly understood clinical challenge. Circulating tumor DNA (ctDNA) enables non-invasive interrogation of epigenomic dynamics through longitudinal sampling, making it particularly attractive for studying resistance mechanisms. However, tumor fraction variability can obscure true epigenomic changes, confounding ctDNA methylation analyses and making robust correction approaches critical to the field. Methods Plasma cell-free DNA methylation was profiled in 50 mCRC patients and 21 age-matched healthy individuals using the Infinium MethylationEPIC v2.0 array. For each patient, paired samples were collected at baseline and at disease progression on standard first-line therapies. A cross-validated Elastic Net model was trained to discriminate baseline mCRC samples from healthy controls, generating a Tumor Fraction (TF) Proxy score. This model was subsequently applied to progression samples for validation. The TF Proxy score was characterized through genome-wide Spearman correlations and Cox proportional hazards regression for time to progression. Results The final model comprised 41 CpG probes and showed strong correlation with deconvoluted epithelial methylation signal ($\rho = 0.79$). Notably, the TF Proxy achieved high discriminative power (AUC = 0.92), outperforming standard epithelial deconvolution (AUC = 0.82) for the detection of baseline mCRC. When applied to the 50 progression samples, baseline tumor epigenetic identity was highly conserved, with the model correctly classifying 88% (44/50) of cases. Genome-wide correlation of the TF Proxy score revealed strong agreement ($|\rho| > 0.80$) with established plasma methylation biomarkers in mCRC, including BCAT1 and SEPT9. Furthermore, patients with a high TF Proxy score experienced a significantly shorter time to progression compared to those with a low TF Proxy (HR = 2.44, 95% CI: 1.32–4.55, p 0.004). Conclusions Our Elastic Net-derived TF Proxy score was validated through accurate identification of progression samples, strong correlation with established plasma methylation biomarkers, and independent prediction of time to progression. Together, these findings support its formal use as a tumor fraction correction tool in differential methylation analyses and as a prognostic biomarker in ctDNA-based epigenomic studies of mCRC. Funding This work was mainly supported by a grant from the Spanish Association Against Cancer in Valencia (PRDVA245884GONZ).

↑ Beginning

# Advancing lung cancer detection: discovery and analytical validation of novel plasma ccfDNA methylation biomarkers

Only Poster | 7. Epigenetic biomarkers

**Main author**: Prof. Carmen Jerónimo (1Cancer Biology and Epigenetics Group, IPO Porto Research Center, Portuguese Oncology Institute of Porto (IPO Porto), Porto, Portugal; · PI)

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**Abstract**:
Background: Lung cancer (LC) remains the most common and the leading cause of cancer related mortality worldwide. This unfavorable epidemiology is largely attributable to the lack of widely implemented screening strategies, resulting in late stage diagnoses and high mortality rates. Aberrant DNA methylation patterns detectable in plasma circulating cell free DNA (ccfDNA) have emerged as promising minimally invasive biomarkers for LC detection. In this study, we identified novel DNA methylation biomarkers through bioinformatic analysis and analytically validated the most promising candidates using clinical samples. Methods: A bioinformatic analysis of The Cancer Genome Atlas (TCGA) methylation datasets was conducted to identify genes exhibiting promoter hypermethylation in LC. Candidate biomarkers were evaluated in lung tumor tissue samples using quantitative methylation specific PCR and subsequently validated in plasma samples using droplet digital PCR in a cohort comprising 80 LC patients and control individuals, including 29 healthy donors and 9 patients with benign pulmonary disease. All samples were obtained from IPO Porto Biobank/Department of Pathology. The study was approved by the institutional review board (Comissão de Ética para a Saúde – CES IPOFG 074/024). Results: Bioinformatic analysis identified 344 genes hypermethylated in LC samples, from which a subset of promising candidates was selected for further evaluation. The selected promoters displayed significantly higher methylation levels in LC patients compared to controls, achieving individual sensitivity values ranging from 58% to 88% and specificity values between 75% to 100%. When combined into a multimarker panel, the assay achieved an overall sensitivity of 87.5% and a specificity of 94.4%, retaining 71.4% sensitivity in early-stage disease. Conclusion: This study demonstrates that a plasma based DNA methylation panel, identified through TCGA data mining and analytically validated by droplet digital PCR in an independent clinical cohort, detects lung cancer with high diagnostic accuracy, including in early stage disease. These findings support the potential clinical utility of plasma based DNA methylation biomarkers as minimally invasive tools for lung cancer detection and their potential future application in screening strategies.

↑ Beginning

# CBX3 as a prognostic biomarker in lung cancer

Only Poster | 7. Epigenetic biomarkers

**Main author**: Dr. Muhammad Waqas (Università degli Studi della Campania Luigi Vanvitelli · 1)

**Abstract**:
Background Lung cancer is among the most common and aggressive malignancies worldwide, marked by a poor prognosis and high mortality. Advances in understanding its molecular mechanisms have highlighted the significance of chromatin-modifying proteins, which are key regulators of gene expression and cellular function. Among these, Chromobox protein 3 (CBX3) has emerged as a potential epigenetic biomarker in lung cancer. Methods A549 and NCI-H1975 cells were cultured in standard conditions. We used molecular and cellular assays to test CBX3 knockdown and overexpression effects on proliferation and survival. Gene expression and epigenetic profiling identified CBX3-linked regulatory pathways. High-throughput screening found compounds that modulate CBX3 activity and block its tumor-promoting effects. Cell viability assay was performed with CCK-8. Basal expression was performed by Western blot and RT-PCR. Results In silico data have indicated high expression of CBX3 in almost all cancer tissues, including lung cancer, and such high expression is associated with a worse prognosis. Notably, RNA sequencing data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression portal (GTEx) show that lung tissues express CBX3 at a significantly higher level than normal lung tissues. Western blot and RT-PCR analyses revealed higher expression of CBX3 in the lung cancer cell lines A549 and NCI-H1975, compared to the normal human lung fibroblast cell line MRC-5. From tissue microarray analysis containing 120 lung tissues samples, CBX3 staining in lung tissues was mainly nuclear. In particular, CBX3 expression is strongly associated with tumor grade, higher histological grade, clinical stage, and tumor size. Conclusions Deciphering the role of CBX3 in lung carcinogenesis could clarify the molecular mechanisms driving tumor aggressiveness. Its identification as a biomarker offers a compelling approach for targeted therapies. Furthermore, the link between CBX3 levels and clinical outcomes underscores its prognostic value, suggesting that inhibiting its function could be a promising treatment plan.

↑ Beginning

# DNA methylation heterogeneity within multiple myeloma patients map to enhancers and repressed Polycomb regions and seems to be associated with survival outcome

Only Poster | 7. Epigenetic biomarkers

**Main author**: Dra. Katarzyna Sokolowska (Independent Clinical Epigenetics Laboratory, Pomeranian Medical University in Szczecin, Szczecin, Poland)

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**Abstract**:
Multiple myeloma (MM) is the second most common hematologic malignancy, typically preceded by monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM). The role of DNA methylation across the plasma cell dyscrasia spectrum still remains insufficiently explored. We performed genome-wide methylation profiling (Infinium MethylationEPIC BeadChip) of CD138 enriched bone marrow plasma cells from patients with MGUS (n = 4) and MM (n = 37) and first assessed heterogeneity of methylation measured as mean of variances of DNA methylation levels per CpG (meanVar). At the genome-wide level, MM patients showed higher methylation variability (MM meanVar = 0.02 and MGUS meanVar = 0.008, p 2.2×10⁻16). During 36-month long follow-up of our study, 12 patients displayed clinically more aggressive disease and died. We analysed methylation differences between patients that died and these that survived (n = 25) and identified 2,671 deferentially methylated CpG sites displaying methylation changes of: $\Delta\beta| \geq 0.05$, |Hedges' g| ≥ 1.0, p ≤ 0.05. Analysis of the genomic localization of the 2,671 CpGs subset in primary B cells (as the closest available proxy for plasma cell precursors) using the Roadmap Epigenomics 15-state core model showed strong enrichment at Bivalent Enhancers ($q = 1.70\times10^{-136}$; OR = 4.6), Active Enhancers ($q = 3.87\times10^{-86}$; OR = 2.8), and Repressed Polycomb regions ($q = 3.40\times10^{-32}$; OR = 2.65), indicating that methylation changes at these regulatory regions may be critical for MM patient survival. The methylation variance at this subset of CpG sites, was significantly lower in patients which survived meanVar: 0.011 vs. 0.027 (in patients who died during follow up), p $2.2\times10^{-16}$. These results suggest that increase of variance at this subset of CpG sites may be implicated in pathology of aggressive MM. Our findings suggest that increase of methylation changes heterogeneity within MM patients at specific subset of CpG sites may be associated with survival outcome and the genomic regions most affected by methylation changes during MM pathology are enhancers and repressed Polycomb regions.

↑ Beginning

# Epigenetic role of HDAC2 in colorectal tumorigenesis

Only Poster | 7. Epigenetic biomarkers

**Main author**: Sr. Biagio Gargiulo (Phd student)

**Abstract**:
Background Colorectal cancer (CRC) is a leading cause of international morbidity and the second highest cause of cancer-related mortality. Incidence risks associated with CRC may be both environmental and behavioral, while disease complexity is characterized by genetic and epigenetic alterations. The up-regulation of the epigenetic regulator HDAC2 is one of most premature events in CRC carcinogenesis. In-depth knowledge of the factors that regulate HDAC2 may reveal the pathological mechanisms related to this protein and the targets for strategies against its functions. Methods We have acquired clinical and pathological details on colorectal cancer patients to gain further insight into the mechanisms of HDAC2-mediated colorectal cancer. RT-PCR analyses were performed to evaluate the expression level of HDAC2 in normal and tumor tissues. In silico analysis were also carried out to estimate the role of HDAC2 expression in CRC tissues compared to normal tissues. Results From RT-PCR data we found that HDAC2 expression in cancer tissue is higher than in normal colon tissue from the same patient. The characterization of high HDAC2 levels in CRC patients were also correlated with clinicopathological features such as tumor progression and stage, presence of metastases, molecular parameters and therapeutic implications. Furthermore, from silico data analysis, we also observed that high HDAC2 expression in CRC is associated with poor prognosis Conclusions HDAC2 may facilitate a series of molecular epigenetic alterations that contribute to the malignant phenotype of CRC. Specifically, HDAC2 may lead to the activation of oncogenes through the deacetylation of histones and non-histone proteins and its overexpression in CRC tissues may lead to disruptions in cellular signaling pathways that promote abnormal cell growth, survival, and invasion. Therefore, HDAC2 presents a promising opportunity as both a biomarker and a therapeutic target, warranting further investigation into its mechanistic roles and potential clinical applications to enhance treatment outcomes for CRC patients. Funding PRIN/PNRR2022 P2022KMP9K “STOP-MPS-Selective pharmacological inhibitors of HDAC6 as novel Therapeutic Option for CNS Pathology in MucoPolysaccharidosiS type IIIA”; PRIN-2022-PNRR-SOLAR-P2022NFCPM,; PRIN-PNRR-2022 Amoebo-switch P2022F3YRF_02 CUP B53D23031620001;

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# A Focused Library Screening Reveals Pan-PKC Inhibitors as Potent Sensitizers for ATRA Therapy in Non-APL AML

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Rafal Skopek (Cardinal Stefan Wyszynski University · Research assistant)

**Co-authors**:

- Institute of Genetics and Animal Biotechnology PAS: Dr. Malgorzata Palusinska (Assistant professor); Karolina Maslinska-Gromadka (PhD student); Prof. Lukasz Szymanski (Professor)

**Abstract**:
Acute myeloid leukemia (AML) is characterized by an accumulation of immature blasts caused by a differentiation block. While all-trans retinoic acid (ATRA) effectively treats AML, non-APL AML subtypes remain stubbornly resistant to ATRA monotherapy. Following a targeted small-molecule screen, we identified pan-protein kinase C (PKI) inhibitors as potent sensitizers capable of unlocking the retinoic acid pathway alone and in combination with ATRA across multiple AML models. Phenotypic evaluations in AML lines (HL-60, KG-1a, NB4) and a non-leukemic control (HEK293). PKI (10 μM) induced massive late apoptosis in HL-60 and NB4 cells. Conversely, the more primitive KG-1a line and HEK293 control maintained high viability under combinatorial PKI and ATRA treatment. In surviving leukemic fractions, this combination robustly induced differentiation, driving marked CD11b upregulation. To translate these findings to a clinical context, we evaluated the PKI and ATRA combination on primary bone marrow blasts derived from a diverse cohort of AML patients. Flow cytometry confirmed that pan-PKC inhibition effectively sensitized these primary samples to ATRA-induced differentiation. Treated blasts exhibited consistent upregulation of mature myeloid markers (CD11b and CD14), downregulation of the stemness marker CD117, and significantly modulated ROS levels. To define the mechanism overcoming ATRA resistance, treated primary samples and cell lines underwent RNA-seq and CUT&Tag sequencing. These analyses confirmed that PKI drives transcriptomic rewiring and epigenomic remodeling. In conclusion, our data indicate that the PKI may successfully reverse the differentiation block in patient samples, shifting leukemic blasts toward a mature physiological phenotype.

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# CBX2 as a Potential Target for Therapeutic Intervention in CRC

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Dra. Nicla Simonelli (University of Campania Luigi Vanvitelli · Post-doc)

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- LUM University: Prof. Carmela Dell'Aversana (Professor)
- Link Campus University: Prof. Nunzio Del Gaudio (Assistant Professor)

**Abstract**:
Background Colorectal cancer (CRC) is driven by the accumulation of genetic and epigenetic mutation with disrupted epigenetic control play a key role in CRC onset and progression. These alterations offer new avenues for potential therapies. Among the epigenetic targets, CBX proteins (CBXs) have emerged as promising candidates. Acting as epigenetic readers, CBXs recognize histone modifications like H3K27me3 and H3K9me3, thereby influencing chromatin conformation and gene expression. Many CBXs are dysregulated in cancer, however CBX2 specifically linked to poor prognosis in CRC. Despite this, the exact molecular role of CBX2 in CRC remains elusive. Results This study investigates the involvement of CBX2 and its domains in CRC progression. We found that CBX2 is overexpressed in CRC primary samples compared to normal tissue. Using a reverse genetic approach, we silenced CBX2 in CRC cells, revealing that its depletion impairs cell growth and induces cell death. Additionally, CBX2 knockdown reduced CRC cell migration, effectively inhibiting their invasiveness. To uncover the molecular mechanisms behind the CBX2-suppressed phenotype, we performed transcriptome-wide analysis. The results showed a downregulation of key gene sets involved in CRC survival pathways, such as eEF2 targets and the TGFβ pathway. Conversely, we observed an upregulation of genes associated with apoptosis, suggesting a shift toward pro-apoptotic signaling in the absence of CBX2. Conclusions Our findings underscore the critical role of CBX2 in CRC carcinogenesis. CBX2 overexpression promotes tumor growth and invasiveness, and small-molecule targeting of CBX2 effectively suppresses CRC cell survival, presenting a promising therapeutic strategy

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# Decoding cellular complexity of the microenvironment of epigenetically driven pediatric high grade gliomas

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Prof. Bozena Kaminska (Nencki Institute of Experimenatl Biology · professor)

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- The Children's Memorial Health Institute: Prof. Wieslawa Grajkowska (Professor); Prof. Katarzyna Kotulska (professor); Dr. Joanna Trubicka (associate professor); Prof. Bozenna Dembowska-Baginska (professor)

**Abstract**:
Pediatric high-grade gliomas (pHGGs) are aggressive brain tumours and the leading cause of deaths in children. They feature unique alterations, such as histone 3 (H3) mutations (K27M, G34R/V) and specific receptor tyrosine kinase fusions, leading to widespread epigenetic dysregulation. Diffuse Midline Glioma (DMG) shows an immunosuppressive and "cold" tumor microenvironment (TME) but underlying tumour-host interactions are poorly understood. We performed a comprehensive analysis of pHGG TME combining single-cell (sc)RNseq with 40 immune cell markers, sc-T cell receptor (TCR) profiling, Visium spatial transcriptomics, multimodal CODEX (Co-detection by indexing) staining to define diverse immune cell types, cellular states and spatial niches that contribute to a cold TME of pHGGs. We profiled >180,000 immune cells from biopsies and corresponding cerebrospinal fluids (CSF) of eight pHGGs. Gliomas with H3 K27M or G34R/V have distinct TME, with distinct contribution of myeloid and lymphoid cells. CD45+ infiltrates in DMG samples predominantly comprise of CD11b+/TMEM119 microglia with less frequent immunosuppressive CD68+Gal3+macrophages, with very few CD3+ T lymphocytes. Microglia expressed transcriptomic programs related to ECM remodeling and angiogenesis, with low expression of chemokines and cytokines, and macrophages displayed phagocytic and immunosuppressive phenotypes. Experiments with co-cultures of wild type or KO DMG K27M cells with microglia uncovered new signaling pathways that could be targeted in small molecules. We attempt to correlate immune features of TME with the presence of onco-histone H3 mutations (K27M, G34R/V). Overall, our data demonstrate distinct abundance and functionalities of immune cells in different epigenetically driven pHGGs which may contribute to the lack of lymphocytes and general immunosuppression.
Studies were supported by the EU Cancer Mission Horizon Europe HIT-GLIO project (101136835) founded by the European Health and Digital Executive Agency.

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# Differential genomic localization of H3 variants in the progression of gastric cancer.

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

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**Abstract**:
Background: Histone proteins are dynamic regulators of chromatin architecture, whose extensive post-translational modifications modulate gene expression. Histone variants H3.2 and H3.3 exhibit tissue-specific genomic distributions and cell cycle–dependent expression. Previous studies have reported elevated H3.2 with reduced H3.3 in gastric cancer. Therefore, this study aims to elucidate the variant-specific dynamics of H3.2 and H3.3 genomic occupancy leading to transcriptional rewiring during tumor progression and metastasis in gastric cancer. Methods: H3.2 and H3.3 were profiled by western blot across gastric cancer cell lines, TGF-β–induced cell line, and an in vivo orthotopic gastric cancer model. Furthermore, transcriptomics and ChIP-sequencing delineated the consequences of differential H3 occupancy on chromatin accessibility and transcriptional reprogramming in both gastric cancer and EMT-induced model. Results: The present study reveals a distinct shift in H3 variants in metastatic gastric cell lines, characterized by upregulation of H3.3 and a simultaneous reduction in H3.2 relative to tumorigenic gastric cell lines. Notably, a gain of the repressive mark H3K9me3 and a loss of the active mark H3K9ac were detected in gastric cancer cell lines, suggesting a repressive chromatin remodelling. Intriguingly, TGF-β–induced gastric cancer cell line has recapitulated this as decreased H3.2 and increased H3.3 relative to untreated parental cells. Transcriptomic analysis further revealed enrichment of metabolic and EMT-associated pathways across gastric cancer cell lines. Consistently, the in vivo orthotopic gastric cancer model showed elevated H3.3 and H3K9ac levels alongside reduced H3.2 and H3K9me3 at metastatic sites compared to primary tumor sites. Furthermore, ChIP-sequencing uncovered distinct genomic occupancy patterns of H3.2–H3K9me3 and H3.3–H3K9ac across tumorigenic, metastatic, and TGF-β-treated gastric cell lines. Integrative analysis of RNA-seq and ChIP-seq revealed that variant-specific redistribution is a determinant of transcriptional reprogramming during gastric cancer progression. Conclusion: Collectively, this suggests variant-specific H3 relocalization as critical epigenetic factor for transcriptional rewiring during cell fate transitions, providing mechanistic insight into the epigenome of tumor growth and metastatic progression in gastric cancer. Funding: Intramural funding from ACTREC, Kharghar.

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# DNA methylation variability is a defining feature of tumor epigenomes with biological and prognostic significance

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Dr. Goran Kungulovski (Fingerprint Diagnostics LLC · Founder and PI)

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**Abstract**:
Background Tumors exhibit substantial cellular and molecular diversity driven by genetic and epigenetic mechanisms. Large-scale profiling efforts have established aberrant DNA methylation as a universal hallmark of cancer. Beyond changes in mean methylation levels, tumor tissues exhibit elevated DNA methylation variability at specific genomic regions within and across tumors. This constitutes a fundamental dimension of cancer epigenomes, reflecting disrupted maintenance of epigenomic states and stochastic drift, which potentially enables adaptation to the microenvironment, phenotypic plasticity, invasion, disease progression, and resistance to treatment. However, the genome-wide organization and functional consequences of DNA methylation variability across cancer types remain incompletely understood. Methods We analyzed paired tumor–normal DNA methylation profiles across 16 cancer types to systematically quantify DNA methylation variability. Pan-cancer DNA methylation variability was validated using complementary statistical approaches. We identified cancer-specific and pan-cancer differentially variable regions and evaluated their associations with genomic features, transcriptional and chromatin regulators, and biological processes. Variability was quantified using three interrelated measures per sample: the proportion of intermediately methylated sites, genome-wide Shannon entropy, and a DNA methylation–based stemness index. Associations with genomic instability, tumor biological features, and clinical outcomes were subsequently assessed. Results Tumor samples consistently exhibited higher DNA methylation variability than matched normal tissues, reflected by increased dispersion and wider interquartile ranges. Pan-cancer variably methylated regions were enriched in heterochromatic domains, depleted at promoter regions, and contained motifs for transcription factors involved in developmental regulation, including Polycomb-associated factors. Elevated DNA methylation variability, captured by higher PIM, entropy, and stemness scores, was associated with increased genomic instability and aggressive tumor features such as lymph node involvement, post-therapy neoplasm events, and elevated hypoxia scores. Importantly, tumors with high DNA methylation variability exhibited significantly worse overall and disease-free survival. Conclusions DNA methylation variability is a pervasive and clinically relevant feature of tumor epigenomes, reflecting epigenetic instability and tumor aggressiveness beyond changes in mean methylation levels. Funding Funded by the European Union under Horizon Europe (project ChatMED Grant Agreement ID: 101159214).

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# Epigenetically associated CD146 heterogeneity defines hybrid epithelial–mesenchymal states in TNBC Cells

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Jadwiga Duda (Student Scientific Group of Epigenetics and Nanotechnology in Medicine EPIGENiusz Jagiellonian University Medical College, Krakow, Poland · student)

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**Abstract**:
Background: Triple-negative breast cancer (TNBC) is characterized by marked intratumoral heterogeneity and high cellular plasticity, contributing to its aggressive behavior and metastatic potential. CD146 (MCAM) is associated with a mesenchymal phenotype, increased migratory capacity, and poor clinical outcome. However, the functional significance of CD146 heterogeneity, particularly the CD146_low fraction, remains unclear. Aim: This study aimed to evaluate whether CD146 expression heterogeneity in the MDA-MB-231 TNBC cell line is associated with distinct epithelial–mesenchymal transition (EMT)-related phenotypes, including key EMT transcription factors. CD146_high and CD146_low subpopulations were isolated from the parental cell line, and CD146 promoter methylation status was assessed in parallel. Methods: CD146 heterogeneity was functionally characterized by separating MDA-MB-231 cells into CD146_high and CD146_low populations using fluorescence-activated cell sorting (FACS). MCAM expression was validated at mRNA (RT-PCR) and protein (Western blot) levels. EMT markers and transcriptional regulators, including CDH2, Vimentin, ZEB1, Snail, and Slug, were analyzed. Cell proliferation and viability were assessed using MTS and crystal violet assays. CD146 promoter methylation was evaluated by bisulfite sequencing. Results: FACS sorting successfully generated distinct CD146_high and CD146_low subpopulations, confirmed at transcript and protein levels. Bisulfite sequencing revealed differences in CpG island methylation within the MCAM promoter between the two groups. EMT analysis showed significantly increased expression of ZEB1 and SNAI1 in CD146_low cells compared to CD146_high cells, while VIM, CDH2, and SNAI2 remained unchanged. Functional assays demonstrated differences in proliferation and viability between subpopulations. Conclusions: CD146_low cells in MDA-MB-231 may represent a hybrid epithelial/mesenchymal state with decoupled transcriptional and structural EMT programs. These findings indicate functionally distinct cellular states within TNBC linked to epigenetic heterogeneity of CD146 promoter methylation. Overall, CD146 reflects not only a phenotypic gradient but also discrete coexisting biological states relevant to tumor plasticity and metastatic potential.

↑ Beginning

# Genome-wide Co-regulation Networks in Aberrant DNA Methylation of Acute Myeloid Leukemia

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Prof. Wolfgang Wagner (RWTH Aachen Medical School · 8)

**Co-authors**:

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**Abstract**:
Epigenetic dysregulation is a defining feature of cancer, and numerous DNA methylation (DNAm) signatures have been proposed as biomarkers for disease stratification. However, the mechanisms that generate these aberrant methylation patterns - and whether they are coherently coordinated across the genome - remain poorly understood. In this study we investigated DNAm alterations in acute myeloid leukemia (AML) by concentrating on CpG sites that are either unmethylated or fully methylated in healthy controls. Although the AML associated DNAm changes were highly heterogeneous and largely patient specific, we identified co-regulated clusters of CpGs that could be assembled into reproducible epigenetic networks across independent cohorts. Multilinear regression models accurately predicted the patient specific DNAm deviations, even for CpGs located on different chromosomes, and the alterations were mirrored on homologous chromosomes. Particularly younger patients with few genomic abnormalites showed increased AML-associated DNAm patterns – and thus the patterns did not reveal prognostic relevance. Strikingly, the same epigenetic networks were recapitulated in a broad spectrum of other malignancies. Acute lymphoblastic leukemia (ALL) exhibited a comparable pattern of co regulation; AML derived models successfully predicted the ALL specific DNAm changes, and the coordination intensified with disease progression. To evaluate the modifiability of these networks, we performed CRISPR guided epigenetic editing at two hub regions in HEK293T cells. In this tumor cell line, the edited sites did not influence the AML associated CpGs, whereas analysis of a public dataset revealed moderate bystander effects at additional AML linked loci after targeted modification. Collectively, our findings demonstrate that the complex, patient specific DNAm landscapes observed in leukemias are not random. Instead, they are orchestrated within interconnected epigenetic networks, highlighting a higher order regulatory layer in cancer epigenomics.

↑ Beginning

# Integrated single-cell genetic and epigenetic profiling reveals desynchronized subclonal evolution in juvenile myelomonocytic leukemia

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Dr. Foued Ghanjati (Department of Pediatric Hematology and Oncology, Children's Hospital, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany)

**Co-authors**:

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**Abstract**:
Background: Juvenile myelomonocytic leukemia (JMML) is a pediatric myeloid malignancy driven by aberrant RAS pathway signaling. Despite established genetic drivers, the relationship between clonal evolution and subclonal epigenetic variation remains incompletely understood. We aimed to determine the co-occurrence of genetic and epigenetic variation at single-cell resolution to test their interdependence during clonal evolution. Methods: Using Tapestri single-cell DNA sequencing and methylation-specific restriction, we developed an integrated platform to jointly interrogate genetic and epigenetic architecture. The panel captured recurrent RAS pathway mutations, secondary cooperating lesions, and DNA methylation loci derived from prior global profiling. Constitutively methylated and unmethylated regions served as internal controls. The assay covered 94 genetic and 147 epigenetic loci. We analyzed bone marrow samples from 12 patients collected at diagnosis. A median of 1457 cells per sample was analyzed with a median sequencing depth of 33 reads per target per cell. Results: Single-cell genotyping identified recurrent evolutionary trajectories, characterized by linear expansion or branching evolution with secondary lesions. Methylation analysis revealed significant cell-to-cell heterogeneity within individual samples and clones, demonstrating that epigenetic diversity is a fundamental JMML feature. Substantial methylation variability was observed between cells within the same genetic clone, indicating that epigenetic diversification is not fully explained by genotype alone. Furthermore, unsupervised analysis recapitulated established bulk methylation classes (low, intermediate, high) at single-cell resolution. These differences were restricted to JMML-informative variable loci, while control regions remained stable. These findings indicate that DNA methylation changes in JMML are structured and context-dependent rather than random. Conclusions: JMML is characterized by clonal evolution coupled to a heterogeneous but organized epigenetic landscape. Integrating single-cell genetic and epigenetic layers provides a high-resolution map of JMML biology, identifying subclones and disease states invisible to conventional genotyping or separate methylation analysis.

↑ Beginning

# Investigating how cancer-specific enhancer rewiring shapes gene regulatory programmes in high-grade serous ovarian carcinoma

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Dr. Marcos Quintela Vázquez (Instituto de Investigación Biomédica de A Coruña (INIBIC) · Investigador Miguel Servet)

**Abstract**:
Background Enhancer reprogramming is a key mechanism of transcriptional dysregulation in cancer, enabling context-specific gene expression programmes that drive tumour progression and therapeutic resistance. Although aberrant enhancer landscapes have been extensively catalogued across cancer types, the functional role of individual regulatory elements remains poorly characterised and their contribution to disease processes is still unclear. High-grade serous ovarian carcinoma (HGSC), the most common and lethal epithelial ovarian cancer subtype, is characterised by genomic instability and a paucity of recurrent protein-coding driver mutations beyond TP53 and DNA repair pathway alterations. This suggests that non-coding regulatory mechanisms, including aberrant enhancer elements, may underlie disease-specific transcriptional programming in HGSC. Methods We integrated publicly available datasets to generate a genome-wide atlas of enhancer landscapes across normal ovarian tissue and HGSC, enabling comparative analysis between healthy and malignant contexts. Enhancers enriched in HGSC relative to normal tissue were identified and linked to putative target genes to define enhancer-gene pairs for downstream investigation. Selected candidates are being evaluated using CRISPR interference and RNA-based perturbation approaches across multiple HGSC models. Results We observed widespread enhancer reprogramming in HGSC compared to normal ovarian epithelium, consistent with subtype-specific epigenomic remodelling. Enhancer-gene associations highlighted a subset of aberrant enhancers linked to increased expression of candidate genes, including the E74 like ETS transcription factor 3 (ELF3). Preliminary perturbation of selected enhancer-gene pairs suggests that these regulatory interactions contribute to HGSC-associated transcriptional changes. Conclusion These findings identify candidate enhancer-gene pairs that represent putative functional regulatory units. These interactions support a framework to dissect how aberrant enhancers contribute to disease-associated gene regulation and downstream phenotypic consequences in HGSC. This framework may further enable investigation of their role in therapeutic response and tumour vulnerability. Funding ISCIII – Miguel Servet Programme

↑ Beginning

# Transposon Derived Neo-Antigens in NSD1 Mutant Head and Neck Cancers

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Dr. Dhriti Tandon (Columbia University · PostDoc)

**Co-authors**:

- Columbia University: Dr. Chao Lu (Associate Professor)

**Abstract**:
Epigenetic dysregulation can initiate the expression of tumor-specific transposons (TEs) or their derived gene products. With the advent of long-read sequencing technologies, we can now mine TEs, which make ~45% of our genome, for neo-antigens that can play a pivotal role in the immune surveillance of malignancies. While neo-antigen coding capabilities of TEs are well known, these have not been studied within the context of cancer specific genotypes. Addressing this is especially relevant for identifying actionable candidates for immunotherapies, as genotypically distinct cancers respond to bespoke treatments. I focus on a sub-type of head and neck, and lung squamous cell carcinoma, which have a distinct NSD1 mutational signature (i.e. loss of function or deletion). NSD1 catalyzes the methylation of H3K36, which recruits DNMT3A to deposit DNA methylation at concordant loci. I plan to annotate novel TE-enriched transcripts associated with NSD1 depletion and assess their coding capabilities as neo-antigens. This approach uses long-read sequencing to annotate new transcripts that are not present in reference annotations, which may not always capture tumor-specific transcription. I hypothesize that NSD1 mutations are associated with anomalous transcription from TEs due to the shifting epigenetic landscape. I assess (Aim1) how NSD1 mutations enhance aberrant transcription from TEs. I discover a greater enrichment of unannotated TE-enriched transcripts, and greater activation of LINE1 and ERV transposons in NSD1 depletion settings. I am currently (Aim2) validating the transcripts at the proteomic levels to assay tumor-specific neoantigens. My findings also suggest the presence of peptides derived from TE-enriched transcripts as HLA bound ligands in NSD1 mutant lines. Lastly, I plan to functionally evaluate whether these peptides are immunogenic by priming them against immature T cells and assaying immune activation. I expect that my study will reveal novel targets for immunotherapies in NSD1 mutant tumors, and inform combinatorial epigenetic and immunotherapy-based treatments.

↑ Beginning

# Validation of the WID-CIN methylation test for detection of Cervical Intraepithelial Neoplasia grade 3 or worse in an independent cohort

To be considered for an Oral Presentation | 1. Clinical cancer epigenetics

**Main author**: Dr. Laura Burney Ellis (Imperial College London · 1)

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- Imperial College London: Dr. Sarah Bowden (2); Dr. Muhummad Habiburrahman (4); Dr. Katerina-Vanessa Savva (5); Dr. Maria Paraskevaidi Paraskevaidi (6); Dr. Christopher Peters (8); Prof. James Flanagan (9); Prof. Maria Kyrgiou (10)
- Imperial College Healthcare NHS Trust: Dr. Jack Tighe (3)
- Aristotle University of Thessaloniki: Prof. Maria Nasioutziki (7)

**Abstract**:
Background Many cervical screening programmes rely on cytology for triage of high-risk human papillomavirus (hrHPV) positive women for referral to colposcopy. DNA methylation has arisen as a potential alternative triage tool, which unlike cytology can be automated and performed on self-samples. The Women's cancer risk IDentification cervical intraepithelial neoplasia (WID-CIN) test is a DNA methylation test comprising 5,000 Cytosine-phosphate-Guanine sites, shown to have promising diagnostic accuracy in initial studies. Methods Women from the UK and Greece (n=247) were recruited over a six year period (2014-2020). Cases were defined as women with histologically confirmed cervical intraepithelial neoplasia grade 3 or invasive disease (n=128) and controls (n=119) had histological and/or cytological evidence of normal cervix. DNA was extracted from exfoliated cervical cells of all participants and subjected to the Human Illumina Infinium MethylationEPIC BeadChip 850K array. The WID-CIN index was applied to the generated beta values and its accuracy assessed at distinguishing case-control status in this cohort. Additionally, we appraised the quality of the index using a validated biomarker toolkit. Results The WID-CIN index achieved an area under the curve (AUC) of 0.898 (95%CI 0.860-0.936) for diagnosis of CIN3+ in all women (N=247). In hrHPV positive women only, the AUC was 0.875 (95%CI 0.793-0.956), with corresponding sensitivity of 0.814 (95%CI 0.716-0.89), and specificity of 0.864 (95%CI 0.651-0.971). In contrast to previous work, the WID-CIN maintained high levels of accuracy in women under 30, reporting an AUC of 0.857 (95%CI 0.762-0.952). In women aged 30 years and over, the WID-CIN test achieved an AUC of 0.906 (95%CI 0.865-0.948). Using the biomarker assessment toolkit, the WID-CIN index appears to be a highly promising translational biomarker. Conclusion The WID-CIN index is a promising biomarker, which may provide effective triage to colposcopy clinics for women who are hrHPV positive. Further validation in large, prospective studies including in screening populations and additionally direct comparison to currently available methods including cytology remain essential next steps. In the era of prolific biomarker generation without sufficient validation, online data sharing, which made this study possible, may provide an important solution for scientific communities willing to collaborate effectively.

↑ Beginning

# Beyond bisulfite: Directly quantifying DNA methylation in the clinic

To be considered for an Oral Presentation | 2. Epigenetic technologies

**Main author**: Sra. Hanne Poulsen (R&D Scientist)

**Abstract**:
A major drawback of current epigenetic methylation analysis methods is that they depend on DNA pretreatment steps like bisulfite conversion or enzymatic digestion. The pretreatment can introduce DNA degradation and analytical bias, and it requires extended processing time. The EpiDirect® technology breaks this dependency. It's a pretreatment-free, quantitative methylation assay running on standard qPCR instrumentation. The technology was first demonstrated to work for assessing MGMT promoter methylation, and here, we will present the next assay, EpiDirect® MLH1, quantifying MLH1 promoter methylation. EpiDirect® can use untreated DNA because the introduction into a primer of proprietary base analogues, the pentabases, can confer preferential binding to methylated DNA. We will explain the scientific background of the technology, show that the analytical performance is comparable to the current gold standard, pyrosequencing, and share how EpiDirect® MLH1 performs against comparator methods in three independent clinical cohorts, testing FFPE-derived DNA from colorectal and endometrial cancers. With a fast and easy means of quantifying DNA methylation directly, future development of EpiDirect® can hopefully broaden the use of epigenetics, both as clinical biomarkers and in biological discoveries.

↑ Beginning

# Cell-specific DNA methylation in human alpha and beta cells regulates gene expression in type 2 diabetes

To be considered for an Oral Presentation | 2. Epigenetic technologies

**Main author**: Dr. Sabrina Ruhrmann (Epigenetics and Diabetes Unit, Department of Clinical Sciences in Malmö, Lund University, Scania University Hospital, Malmö, Sweden · Assistant Researcher)

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**Abstract**:
Background: The number of people affected by type 2 diabetes (T2D) is rapidly increasing worldwide. Genetics only explains a modest proportion of the heritability of T2D. However, studies performed during the last decades have identified epigenetic alterations in tissues from individuals with T2D compared with controls. Those epigenome wide association studies of human pancreatic islets have often been performed in bulk tissues and lack the methylomes of individual cell types like e.g. beta and alpha cells in the context of T2D pathogenesis. Methods: Here we set out to investigate methylomes and transcriptomes of sorted pancreatic beta and alpha cells using whole genome bisulfite sequencing (WGBS) and RNA-sequencing (RNA-Seq). Results: We identify 22,544 differentially methylated regions (DMRs), that are annotated to 7975 genes between pancreatic beta and alpha cells annotated to e.g. cell specific genes like INS and GCG , encoding insulin and glucagon, respectively. Active re-writing of the epigenetic signature at these loci by applying CRISPR-dCas9-DNMT3a and CRISPR-dCas9-TET1 based epigenetic editing in beta cells leads to increased DNA methylation at the targeted INS locus and decreased DNA methylation at the targeted GCG locus. Both approaches result also in corresponding alterations in INS and GCG expression and content in beta cells indicating the identification of causal epigenetic changes at these loci. Furthermore, the identified DMRs in beta and alpha cells, that are associated with pre-T2D/T2D, overlap with 18% and 12% of T2D associated risk genes, respectively. We also summarize all the acquired data in the web tool (https://alpha-beta-methylome.serve.scilifelab.se/app/alpha-beta-methylome/) and create a resource for exploring T2D, age and sex associations on DNA methylation. Conclusion: This study provides novel insights into causal epigenetic changes occurring in cell types relevant to type 2 diabetes, particularly at important signature genes.

↑ Beginning

# FLEA-ChIP: A differentiated platform for challenging clinical samples

To be considered for an Oral Presentation | 2. Epigenetic technologies

**Main author**: Dra. Marina Ruiz-Romero (Centre de Regulació Genòmica · Researcher)

**Co-authors**:

- Centre de Regulació Genòmica: Sra. Zighereda Ogbah (Technician); Prof. Roderic Guigó (Principal investigator); Dra. Sílvia Pérez-Lluch (Staff Scientist)

**Abstract**:
Epigenetic alterations are increasingly recognized as clinically relevant biomarkers for cancer diagnosis, patient stratification, and treatment-response monitoring. While DNA mutations and DNA methylation are already widely used in precision oncology, chromatin-based biomarkers remain underexplored in clinical settings, largely because genome-wide profiling of histone modifications typically requires substantial input material and complex experimental workflows. This limitation is particularly relevant for low-input clinical samples, where biological material is scarce and standard chromatin immunoprecipitation approaches are difficult to implement. Here, we present FLEA-ChIP, an ultra-low-input chromatin profiling platform designed to enable robust genome-wide mapping of histone marks from clinically relevant samples. FLEA-ChIP aims to provide a time- and cost-effective solution for profiling chromatin states in limited material, starting from as few as 500 cells, and supporting the identification of epigenetic biomarkers associated with disease state, tumor regulatory programs, and therapeutic response. In addition to low-cell-number samples, FLEA-ChIP has the potential to be adapted to cell-free chromatin applications, including liquid biopsies, further expanding its clinical utility for minimally invasive disease monitoring. We are also optimizing the protocol toward single-cell applications, with the aim of increasing resolution and enabling the study of cellular heterogeneity. By focusing on histone modifications, FLEA-ChIP captures a dynamic and functionally informative layer of gene regulation that complements existing genomic and DNA methylation-based assays. We will discuss the rationale behind FLEA-ChIP, its potential integration into clinical biomarker discovery pipelines, and its relevance for precision medicine. By enabling genome-wide chromatin profiling from ultra-low-input samples, FLEA-ChIP has the potential to expand the clinical utility of epigenomics and open new opportunities for translating chromatin biology into actionable diagnostic and therapeutic strategies.

↑ Beginning

# The 12 o'clock assay: an optimized dodecaplex droplet digital PCR assay for improved DNA methylation quantification and epigenetic clock-based age-predictions

To be considered for an Oral Presentation | 2. Epigenetic technologies

**Main author**: Ilef Hchaichi (Laboratory for Genomics, Foundation Jean Dausset – CEPH, Paris, France · Ph.D. Student)

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**Abstract**:
DNA methylation is one of the best biomarkers of aging, strongly correlated with chronological age and used in epigenetic clocks to predict age, which can also reflect health status. Although new age-prediction models are developed to support novel applications, DNA methylation quantification rely on a limited number of techniques presenting several limitations and biases, which decrease the accuracy and reproducibility of age predictions. To address this, we here developed a new droplet digital PCR (ddPCR) assay format accurately quantifying DNA methylation, based on three LNA-containing hydrolysis probes to detect either the methylated or unmethylated allele. We optimized a 12-plex assay quantifying simultaneously methylation in six CpGs (ASPA, C1orf132, CCDC102B, EDARADD, ELOVL2 and FHL2), which is strongly correlated with age ($-0.83 > r > 0.85$) when applied to 351 blood samples from individuals aged from 0 to 95 years. Several age-prediction models were developed using four different statistical approaches that showed good prediction performances on testing set samples ($0.933 R^2 0.973$, $5.18 > MAE > 3.18$, and $7.27 > RMSE > 4.71$). This assay significantly improves and simplifies DNA methylation quantification accross multiple targets for age-prediction models and supports numerous applications, from forensics to biomedical research.

↑ Beginning

# Androgen stimulation rapidly reorganizes temporal 3D genome and epigenome states to trigger AR-mediated transcription in prostate cancer.

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Prof. Susan Clark (Garvan Institute of Medical Research · Lab Head)

**Co-authors**:

- Garvan Institute of Medical Research: Sra. Elyssa Campbell (PhD student); Dr. Amanda Khoury (Postdoctoral Fellow)
- SAiGENCI: Dr. Joanna Achinger-Kawecka (Lab Head); Sra. Geraldine Laven-Law (research assistant)

**Abstract**:
BACKGROUND Androgen receptor (AR)-mediated transcription drives prostate cancer progression and remains a critical therapeutic target. AR activation by androgens triggers nuclear translocation, DNA binding at AR regulatory elements (AREs) and transcription initiation. This process involves co-factors such as FOXA1, epigenetic modifications, and 3D chromatin interactions. However, the temporal coordination of these events during AR-mediated transcription remains poorly understood. RESULTS Using a time-course androgen stimulation model in prostate cancer cells, we integrated temporal multi-omics data within the MOFA+ framework to dissect AR-mediated transcriptional dynamics. Our analysis revealed that rapid AR binding at gene promoters is crucial for initiating nascent transcription of AR target genes. We identified H3K27 acetylation at flanking AREs, pre-marked by constitutive FOXA1 binding, which is a prerequisite for AR recruitment. Additionally, androgen stimulation induced a temporal early shift in nascent transcription from MYC to AR target genes, likely mediated by transient disruption of 3D chromatin interactions. Finally, we performed CRISPR-based inhibition on key AR binding sites identified in the MOFA+ model and established their functional consequences on androgen-induced transcription. CONCLUSIONS These findings uncover a dynamic reorganization of 3D chromatin structure and transcription factor binding post-androgen stimulation, resulting in distinct temporal epigenomic states. This work provides new insights into the mechanisms governing AR-mediated transcriptional activation in prostate cancer and highlights the interplay between MYC and AR in regulating transcription in hormone-driven cancers.

↑ Beginning

# Biomolecular condensation of E2A-PBX1 oncoprotein drives chromatin organization and leukemogenesis in B-ALL

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Sra. Marta López García (Josep Carreras Leukaemia Research Institute (IJC) · PhD student)

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- Centre for Genomic Regulation (CRG): Sra. Marta López-Osias
- Max Planck Institute for Molecular Genetics: Dr. Denes Hnisz

**Abstract**:
B-cell Acute Lymphoblastic Leukemia (B-ALL) is one of the most common and aggressive forms of leukemia, particularly affecting children and young adults. A recurrent form is caused by a chromosomal translocation t(1;19), which results in the formation of the E2A-PBX1 chimeric transcription factor. While the oncogenic potential of E2A-PBX1 has been characterized, the mechanisms by which it alters 3D chromatin architecture and transcriptional regulation in B-ALL remain unclear. Here, we aim to uncover how E2A-PBX1 reshapes genome organization and drives leukemic transcriptional program. Using genomic studies in an E2A-PBX1-driven B-ALL cell line, we show that E2A-PBX1-bound regions form long-range hubs of accessible chromatin, leading to widespread gene activation. Notably, in silico analysis of E2A-PBX1 amino acid sequence revealed a large intrinsically disordered region (IDR) at the N-terminal part of the fusion protein. We showed that this IDR is able to phase separate in vitro in a concentration, salt and temperature-dependent manner. In cells, E2A-PBX1 forms nuclear condensates and its IDR is able to drive light-induced condensate formation. By performing mutagenesis on the IDR sequence, we demonstrated that this condensation is sensitive to mutations in aromatic residues (AroLite-mutant), which are essential for efficient phase separation. Functionally, alteration of the E2A-PBX1 condensation properties in the AroLite-mutant impairs the regulation of key transcriptional leukemic targets and its ability to immortalize hematopoietic progenitors. We propose that E2A-PBX1 condensation may participate in activating leukemic transcriptional program through remodeling of 3D chromatin organization. Targeting the E2A-PBX1 condensates might offer potential new therapeutic opportunities for treating B-ALL.

↑ Beginning

# Chromatin dysregulation in uterine leiomyomas: insights from CRISPR engineered model cell lines

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Dr. Kristiina Rajamäki (1 Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland · Senior researcher)

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**Abstract**:
Background: Uterine leiomyomas (ULs) are extremely common benign neoplasms of the uterine wall muscle layer, myometrium, affecting up to 80% of premenopausal women; 15-30% display symptoms such as excessive bleeding, pain, subfertility and recurrent pregnancy loss. The only curative treatment is surgery, often removal of uterus by hysterectomy, posing a great burden on women's health and economy. ULs can be divided into molecular subclasses based on mutually exclusive genetic driver alterations in MED12 (70% of ULs), HMGA1/2, FH, and subunits of the SRCAP chromatin remodeling complex depositing histone variant H2A.Z onto chromatin. Our group recently discovered that across the tumour subclasses, UL driver mutations cause deranged chromatin regulation particularly at bivalent genomic regions poised for rapid activation and commonly marked by H2A.Z (Berta Nature 2021). How these regulatory genome alterations emerge remains poorly understood. Methods: To identify the immediate effects of UL driver mutations, we created four CRISPR engineered UL model cell lines from immortalized human myometrial smooth muscle cells: MED12 p.G44D hotspot mutation and homozygous loss-of-function mutations for three SRCAP complex members (YEATS4, DMAP1, ZNHIT1). Mutant and wild-type cell lines underwent phenotypic characterization, Assay for Transposase-Accessible Chromatin (ATAC)- and RNA sequencing. Results: The cell lines retained smooth muscle phenotype. Samples from each mutant cell line clustered together and separate from wild-type cells based on gene expression and differentially accessible genomic regions (DARs). SRCAP complex gene mutant cell lines more closely resembled each other in RNA-seq clustering, yet 78 of their 195 shared differentially expressed genes were also shared with MED12 cell line. The most significant impact of DARs on gene expression was observed at the same myometrial chromatin state annotation in all four mutant cell lines. Results will be further compared to our existing genomic data from primary tumours (Berta Nature 2021). Conclusions: Engineered UL cell models allow us to distinguish the immediate biological effects of UL driver mutations – likely representing key early events in tumorigenesis - from selection mediated and stochastic changes evolving over time in UL tissue. Funding: Research Council of Finland, Jane and Aatos Erkko Foundation, iCANDOC doctoral program

↑ Beginning

# Context-dependent recruitment of co-factors directs RUNX1 activity in normal and leukemic cells

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Dr. Dimitra Dimou (University of Oxford, MRC Weatherall Institute of Molecular Medicine, Oxford, United Kingdom · Academic Clinical Fellow in Histopathology, Addenbrooke's Hospital, Cambridge)

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- Hugh and Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Department of Immunology and Inflammation, Imperial College London, London, United Kingdom: Dr. Nicholas Crump (Associate Professor in Myeloma Research)

**Abstract**:
Background: Aberrant transcription factor (TF) activity is a major driver of diseases such as leukemia. Many TFs have dual activity functioning both as activators and repressors, but the precise mechanisms of this dual activity are unclear. RUNX1 is a key hematopoietic TF, frequently mutated in leukemia and implicated in both activation and repression of gene targets. However, despite its crucial role in hematopoiesis, it is unknown how RUNX1 mediates this dual role at specific loci or whether this has implications for leukemia-associated RUNX1 mutations. Methods: An established anchoring system was employed to investigate the recruitment of chromatin proteins by Runx1. Runx1 was fused to the DNA-binding domain of the Tet repressor and targeted to a gene-poor region in a neutral chromatin context in mESCs, in which it is not endogenously expressed. Runx1 interaction partners were then identified using chromatin immunoprecipitation (ChIP). This system was also used to map the regions of Runx1 mediating these interactions. To assess the functional consequences of Runx1 activity on chromatin regulation, ChIP, siRNA-mediated knockdown, and RNA-seq were performed in leukemia cell lines and primary patient samples. Results: We found that RUNX1 alone is sufficient to recruit key chromatin proteins, both co-activators and co-repressors, including Trithorax group (TrxG) and Polycomb group (PcG) complexes. RUNX1-mediated recruitment of co-activators and co-repressors at endogenous loci is dependent on the factors and histone marks that are already present, with RUNX1 “amplifying” existing activating or repressive signals. Further, we showed that C-terminally truncated RUNX1 mutant proteins, including the RUNX1-RUNX1T1 fusion protein, fail to recruit PcG complexes, and that acquisition of PcG mutations in RUNX1-RUNX1T1 AML (associated with worse prognosis) results in deregulation of key target genes, including CCND1. Conclusions: Our results provide a mechanistic understanding for how a TF “amplifies” existing activating or repressive signals and can “switch” between co-factors to selectively drive activation or repression at target sites; and demonstrate how disease-associated mutations disrupting the interaction between a TF and its co-factors can promote leukemogenesis.

↑ Beginning

# Dialysis-Associated Epigenetic DNA Methylation Signatures Linked to Inflammation in Hemodialysis Patients

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Sra. Hira Syeda (University of Saskatchewan · Doctoral Candidate)

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**Abstract**:
Background: Hemodialysis patients are repeatedly exposed to dialysis membranes over prolonged periods. Differences in membrane material and surface chemistry can stimulate immune activation and contribute to chronic inflammation. Although dialysis membranes are central to treatment, current assessments rely mainly on clearance measures and inflammatory biomarkers, which do not reflect cumulative biological effects of membrane exposure. Epigenetic mechanisms, particularly DNA methylation, offer a stable way to capture these biological responses and provide new insight into dialysis-associated inflammation and patient well-being. Methods: Paired pre- and post-dialysis blood samples were collected from hemodialysis patients treated with the same dialysis membrane. Genome-wide DNA methylation profiling was performed following genomic DNA extraction and bisulfite conversion. Differentially methylated regions were identified using bioinformatic workflows, followed by functional pathway enrichment and protein–protein interaction network analyses. Inflammatory and complement biomarkers were quantified using multiplex immunoassays. Membrane–protein interactions were assessed by visualizing fibrinogen adsorption within dialysis membrane layers using in situ synchrotron radiation tomography at the Canadian Light Source. Results: Thousands of differentially methylated regions were identified between pre- and post-dialysis samples. Differential methylation was observed in genes involved in inflammatory signaling, vascular regulation, immune modulation, and cellular stress responses. Functional enrichment analysis highlighted pathways related to cytokine activity, immune regulation, vascular remodeling, transcriptional control, and neurobiological signaling. Protein network analysis identified central regulatory hub genes, including CDKN2A, CXCL12, TWIST1, and WNT5A. Post-dialysis samples showed a predominance of hypermethylation in inflammation-associated pathways. Elevated inflammatory biomarkers, together with increased fibrinogen adsorption across membrane layers, supported a link between blood–membrane interactions, inflammatory activation, and epigenetic dysregulation. Conclusion: Hemodialysis membrane exposure is associated with distinct DNA methylation patterns in inflammatory, vascular, and immune regulatory pathways in human patients. These epigenetic changes reflect cumulative biological responses to repeated dialysis exposure that are not captured by conventional clearance measures or transient inflammatory biomarkers. Epigenetic profiling may enhance evaluation of membrane biocompatibility and improve understanding of dialysis-associated inflammation. Funding: Supported by the Saskatchewan Health Research Foundation, the Natural Sciences and Engineering Research Council of Canada, and the University of Saskatchewan.

↑ Beginning

# DNA methylation loss impairs nuclear architecture and promotes chromosomal instability through heterochromatin relaxation

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Serena Gargano (Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy · PhD student)

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- Matière et Systèmes Complexes, CNRS, Université Paris-Cité, France: Jean-Baptiste Manneville (Researcher)

**Abstract**:
Background: DNA methylation is an epigenetic modification that regulates chromatin structure and function by promoting heterochromatin. Global loss of DNA methylation at gene-poor repetitive sequences - the most abundant genomic regions, mainly heterochromatic - is a hallmark of genomic instability and cancer. Importantly, heterochromatin displays a defined three-dimensional organization within the nucleus and, together with the nuclear lamina, regulates nuclear stiffness. Thus, we hypothesized that global DNA hypomethylation, by affecting heterochromatin, compromises nuclear architecture and mechanical properties. This study explores this connection, proposing a novel mechanism by which DNA hypomethylation promotes genomic instability and tumor formation. Methods: Our study employed the non-tumor immortalized RPE-1 cell line engineered to induce DNA hypomethylation. Upon DNA methylation loss, alterations of nuclear morphology and nuclear envelope composition were assessed by immunofluorescence and Western blot analyses. Nuclease sensitivity assay was performed to assess changes in chromatin accessibility. Also, nuclear mechanical properties were tested using mechanosensors and biophysical approaches. In addition, wound-healing assay allowed us to evaluate the effects of DNA hypomethylation on cell migration. Finally, chromosome nuclear positioning and mitotic segregation defects were examined by FISH and cytogenetic analyses. Results: We observed that hypomethylated nuclei exhibit increased size and higher blebbing, associated with reduced levels and impaired localization of key proteins of the nuclear envelope (LaminB1 and LBR), suggesting its compromised integrity. Biophysical measurements further reveal decreased nuclear membrane tension and stiffness, consistent with enhanced nuclear deformability. We also observed altered cell migration capability. Furthermore, hypomethylated cells accumulate mitotic defects and acquire aneuploidy, with chromosomal gain or loss preferentially affecting originally highly heterochromatic chromosomes, underscoring the critical role of DNA methylation in supporting chromosome segregation. Notably, DNA hypomethylation induces global heterochromatin relaxation, indicating a widespread chromatin reorganization within the nucleus underlying these observed phenotypes. Conclusions: Altogether, these findings demonstrate that DNA hypomethylation disrupts heterochromatin organization, leading to alterations of nuclear physical properties and impaired chromosome dynamics. Overall, this work highlights a novel mechanism by which DNA methylation preserves genome stability by maintaining nuclear architecture, proposing DNA hypomethylation as a potential oncogenic event. Funding: This work was supported by French BioImaging and Eurostart2025.

↑ Beginning

# Dynamic changes of discordant methylation patterns at adjacent CpG sites are associated with pathogenesis of chronic lymphocytic leukemia

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Sr. Jan Zadworny (Pomeranian Medical University in Szczecin)

**Co-authors**:

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**Abstract**:
Background: We have recently shown that two CpG sites spaced less than 50bp can acquire different methylation status and the methylation status of those CpG sites is tissue specific [1]. Here, we investigate how this phenomenon is altered during the pathogenesis of chronic lymphocytic leukemia (CLL) as well as investigate the biological and clinical consequences of changes in the methylation status of discordantly methylated CpG sites. Methods: The discordant methylation patterns were analyzed using EPIC microarrays from 114 CLL patients and correlated with RNA-seq expression profiling data as well as clinical outcomes, including time to treatment and overall survival. Methylation profiling data from B-cells populations were used as reference. Results: We showed that methylation patterns at discordantly methylated loci undergo dynamic changes during CLL pathology, with methylation patterns at some loci changing completely from the ones observed in B-cells. However, we found no association between methylation levels at these loci and the expression of corresponding genes. For a subset of loci, there was a strong association between methylation status change and time to treatment and overall patient survival. Conclusions: Discordant methylation patterns undergo dynamic changes in CLL and could be used as biomarkers of disease progression. However, given the lack of association of studied methylation changes with gene expression, further research is required to elucidate the underlying biological mechanisms of these epigenetic alterations, especially in the context of the clinical utility of these methylation changes as biomarkers of clinical outcomes. Funding: This work was funded by grant OPUS22 from Polish National Science Centre (grant number: 2021/43/B/NZ2/02979) [1] Taryma-Leśniak, Olga, et al., Epigenetics & Chromatin 17.1 (2024): 30.

↑ Beginning

# Dynamic epigenetic regulation of BCLAF1 splicing in acute myeloid leukemia

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Alternative splicing dysregulation is increasingly recognized as a key contributor to cancer development and tumor progression. Among RNA-processing regulators, BCL2-associated transcription factor 1 (BCLAF1) plays a multifaceted role in essential cellular processes, including transcriptional regulation, apoptosis, and cellular stress responses. However, its involvement in hematological malignancies and its contribution to acute myeloid leukemia (AML) pathogenesis remain incompletely understood. This study aims to investigate the regulation of BCLAF1 splicing in AML, focusing on the biological consequences of isoform imbalance and its potential reversibility through epigenetic modulation. Methods PCR, WB, and qPCR analyzed BCLAF1 alternative splicing and quantified isoforms. ChIP-qPCR assessed epigenetic regulation. Co-IP examined protein–protein interactions. RIP analyzed BCLAF1-associated RNAs. MS/MS identified protein interactors. shRNA was used to knock down DNMT3A and DNMT3B. Results Two distinct BCLAF1 isoforms were identified in AML cell lines: a full-length isoform associated with oncogenic activity and a short-length isoform with tumor-suppressive properties. A marked imbalance between these isoforms was observed in leukemic cells, indicating disrupted splicing regulation. Treatment with specific epi-drugs were able to restore the physiological isoform ratio, demonstrating that these alterations are reversible and dynamically regulated. Functional experiments revealed that DNMT3A and DNMT3B contribute to the control of BCLAF1 splicing and are integrated into a broader epigenetic network involving HDAC1 and MRG15. Mass spectrometry further supported the existence of a protein interaction network linking epigenetic regulators to splicing machinery, suggesting coordinated control of BCLAF1 isoform expression. Conclusions This study uncovers a previously unrecognized epigenetic regulatory axis that governs BCLAF1 alternative splicing in AML. The opposing functions of its isoforms reveal a critical imbalance between oncogenic and tumor-suppressive pathways in leukemia. Importantly, our findings show that epigenetic modulation can restore physiological splicing patterns, highlighting the therapeutic potential of targeting epigenetic regulators in AML. Notably, preliminary data suggest that a similar mechanism regulating BCLAF1 splicing may also operate in colorectal cancer (CRC), pointing to a broader relevance across different tumor types.

↑ Beginning

# Epigenetic and Plasma C-Reactive Protein in Relation to Regional Brain Structure and Psychosis Outcomes

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Systemic inflammation and structural brain alterations have both been shown to be predictors of psychosis among youth at clinical high risk for psychosis (CHR-P). Epigenetic markers, such as DNA methylation (DNAm), provide proxies for inflammation. While elevated C-reactive protein (CRP) has been associated with reduced gray matter volume (GMV) and psychosis, there have been no studies comparing plasma and epigenetic CRP markers in relation to structural brain changes and psychosis. This study tested the relationships between CRP (derived from plasma and DNAm) and regional GM volumes, as well as attenuated positive symptoms, among youth at CHR-P and healthy controls (HC). Methods Participants were recruited for the North American Prodrome Longitudinal Study Phase 2 (NAPLS-2; 2008–2013). Plasma CRP markers were quantified via multiplex immunoassay, and epigenetic CRP markers were derived from Illumina 450k array data. GMV of the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), and hippocampus was extracted from 3-Tesla MRI scans. Attenuated positive symptoms were assessed with the Scale of Prodromal Symptoms (SOPS). Structural equation model (SEM) estimated associations among plasma and epigenetic CRP measures, GMV, and symptom severity, adjusting for demographic, clinical, and intracranial volume covariates, with site-level clustering and bias-corrected bootstrapping estimates. Results This study included 165 CHR-P and HC participants (67% female; mean age = 19.05 years). Several regions were significantly associated with epigenetic CRP but not with positive symptom severity, while others were associated only with positive symptom severity and not with epigenetic CRP. Only one regional volume showed significant associations with both measures. Higher epigenetic CRP measures were significantly associated with reduced caudal ACC (β = −0.08; 95% CI: −0.13 to -0.01), which in turn predicted greater positive symptom severity (β = −0.26; 95% CI: −0.43 to −0.15). No significant associations were observed for plasma CRP. Conclusions Epigenetic, but not plasma, CRP markers were associated with reduced cACC volume, which, in turn, was linked to greater positive symptom severity. Consistent with prior findings, epigenetic CRP may provide a more reliable signature of chronic inflammation that may be reflected in structural brain changes linked to psychosis.

↑ Beginning

# Epigenetic Regulation of CD146 Reveals Phenotypic Plasticity and Stable Subclones in TNBC Cells.

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background: Triple-negative breast cancers (TNBC) exhibit marked phenotypic heterogeneity, contributing to tumor progression and therapy resistance. CD146, a marker associated with epithelial–mesenchymal transition (EMT) and aggressive tumor behavior, shows variable expression in TNBC cells and is epigenetically regulated by promoter DNA methylation. However, the relationship between methylation heterogeneity and CD146-defined cellular states remains unclear. In the MDA-MB-231 cell line, heterogeneous CD146 promoter methylation suggests the presence of distinct cellular subpopulations. This study aimed to determine whether CD146 heterogeneity reflects reversible phenotypic plasticity, stable epigenetically defined subpopulations, or both, and to assess the relationship between promoter methylation and CD146 expression. Methods: Cells were labeled with anti-CD146 and sorted by fluorescence-activated cell sorting (FACS) into CD146-low and CD146-high fractions using two strategies: bulk sorting ($10^6$ cells) and stringent low-cell-number sorting (10 cells) to enrich distinct subpopulations. DNA methylation across 22 CpG sites within the CD146 promoter was analyzed by bisulfite sequencing. CD146 expression was assessed by RT-PCR and Western blot. Results: Bulk-sorted populations showed pronounced phenotypic plasticity: CD146-low cells increased to 30–60% CD146-positive cells, while CD146-high cells decreased to ~70% over subsequent passages, indicating reversible state transitions. In contrast, low-cell-number–derived populations maintained stable CD146 expression profiles across passages, supporting the existence of stable epigenetic states. Bisulfite sequencing identified four differentially methylated CpG sites near the transcription start site. CD146-low subclones displayed relative hypermethylation at these loci, suggesting that focal promoter methylation contributes to stable CD146 repression. Differential CD146 expression was consistently confirmed by FACS, RT-PCR, and Western blot in independently derived CD146-low and CD146-high clones, supporting a link between promoter methylation and the maintenance of distinct cellular states. Conclusions: CD146 heterogeneity in MDA-MB-231 cells is driven by dynamic phenotypic plasticity and the presence of stable epigenetically defined subpopulations. Promoter methylation contributes to CD146 regulation and may facilitate the emergence of functionally distinct tumor cell states. These findings support a model in which epigenetic reprogramming and clonal selection jointly shape intratumoral heterogeneity in TNBC, with potential implications for therapeutic resistance and targeted treatment strategies.

↑ Beginning

# hsa-mir-98-5p – a new player in classic Hodgkin lymphoma development

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Aberrant miRNA expression has been increasingly recognized as a key regulatory mechanism in tumor biology. In our previous study, we used small RNA-seq validated by RT-qPCR and identified hsa-miR-98-5p to be recurrently overexpressed in classic Hodgkin lymphoma (cHL) cell lines and patient-derived tumor cells as compared to germinal center B-cell (GCB) and non-Hodgkin lymphoma (NHL) cell lines. Therefore, we hypothesize that hsa-miR-98-5p plays an yet unrecognized oncogenic role in cHL. Methods To decipher its role, we knocked-down miR-98-5p in 4 cHL cell lines using a MirZip vector and assessed the effects of its inhibition on cellular phenotypes using GFP competition, cell viability and apoptosis assays. To identify putative target genes of hsa-miR-98-5p, AGO2-RNA immunoprecipitation and sequencing (AGO2-RIP-seq) was performed on 3 cHL cell lines. Candidate target genes were selected based on the presence of the canonical miR-98-5p seed sequence binding site within their 3'UTR and an AGO2 enrichment score > 4. Finally, expression levels of the identified target genes and their DNA methylation level were analyzed in HL, NHL, and GCB-derived cell lines using RNA sequencing and Illumina HumanMethylation450 BeadChip (EGA repository: EGAS50000000627), respectively. Results Knock-down of miR-98-5p expression resulted in reduced rate of cellular proliferation in 3/4 analyzed HL cell lines (p 0.001), due to both higher apoptosis rate (3/3 cell lines, p 0.005) and reduced cell viability (2/3 cell lines, p 0.05). Using AGO2-RIP-seq we identified 37 genes potentially targeted by miR-98-5p. Within this group, E2F5 (E2F Transcription Factor 5) and BTG2 (B-Cell Translocation Gene 2) were significantly downregulated (p 0.05) in HL cell lines compared to NHL and GCB cell lines, while CDKN1A, CRY2, TRIB1 and TNFSF9 were downregulated in HL cell lines relative to GCB controls (p 0.05). Only in the BTG2 regulatory region we identified a region (chr1:203,304,400-203,305,100; hg38) of elevated methylation in cHL (mean 58%) compared to NHL cell lines (mean 18%). Conclusions Our results suggest that miR-98-5p functions as a previously unrecognized oncomiR in cHL development, contributing to the silencing of BTG2, CDKN1A, CRY2, E2F5, TRIB1 and TNFSF9 genes. Funding The Polish National Science Centre grant: 2020/39/B/NZ2/01004

↑ Beginning

# Hypoxia-driven lipid rewiring of microglia cells reveals a targetable epigenetic vulnerability in high-grade gliomas

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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- 3P-Medicine Laboratory, Medical University of Gdansk, Gdansk, Poland: Prof. Jakub Mieczkowski (Group Leader)

**Abstract**:
Background. Hypoxia rapidly alters gene expression to allow cellular adaptation to challenging conditions and support tumour growth. Hypoxia also affects the chromatin structure by changing histones and DNA modifications. High-grade gliomas (HGG), such as adult glioblastoma (GBM) or paediatric HGGs (pHGG) are aggressive, deadly primary brain tumours for which there is no effective treatment. The tumour microenvironment of HGGs is highly heterogeneous, with infiltration of glioma-associated microglia and macrophages (GAMs) and the presence of necrotic, hypoxic regions. The mechanisms through which hypoxia alters functions of GAMs remain poorly understood. Methods and results. We show that hypoxia modulates the expression of myeloid markers in the opposite ways: upregulates the monocytic marker Lgals3 and downregulates the microglial markers P2ry12 and Tmem119 in GAMs in vitro and in vivo, as shown using human and mouse GBM single-cell transcriptomics datasets, in vitro co-culture models and CODEX multi-marker proteomic analysis in human adult and paediatric HGG tumour sections. Hypoxia dysregulates additional GAM subtype markers through changes in accessible chromatin, as determined with ATACseq. While hypoxia alone decreases the overall chromatin accessibility at gene promoters, exposure to glioma cells under hypoxic conditions leads to both increases and decreases of chromatin accessibility in promoters of microglial cells. Moreover, changes in histone acetylation are responsible for Lgals3 upregulation and lipid accumulation in GAMs in hypoxic conditions. This scenario is additionally enhanced in H3K27-altered diffuse midline gliomas, where the presence of H3K27M oncohistone additionally strengthens the lipid accumulation in microglia. Conclusions. Overall, our data highlights the importance of hypoxic stress as a strong intratumoral regulator of epigenome, which influences myeloid cell functions. Response to hypoxia is additionally rewired by the expression of the oncohistone H3K27M, which aligns with our recent work showing that histone deacetylase inhibition reduces H3K27M protein levels in pHGGs and normalises lipid accumulation in hypoxic myeloid cells, which may open a new therapeutic window for dual targeting of HGG features. Funding. Studies were supported by the National Science Centre (Poland) grants (2019/33/B/NZ1/01556 and 2024/54/E/NZ3/00480) and the EU Cancer Mission Horizon Europe HIT-GLIO project (101136835) founded by the European Health and Digital Executive Agency.

↑ Beginning

# Identification of Parkinson’s disease-associated regulatory variants in human dopaminergic neurons reveals modulators of SCARB2 and BAG3 expression

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background A hallmark of Parkinson’s disease (PD) is the degeneration of midbrain dopaminergic neurons (mDANs). Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with PD, but causal variants and mechanisms remain unknown. Many PD-associated SNPs reside in regulatory regions, where they may disrupt transcription factor binding sites (TFBS) and alter gene expression. Methods To assess how non-coding PD SNPs affect gene regulation in mDANs, we identify variants predicted to alter TF binding and functionally validate their effects in a cell type-specific context. We integrate time-series transcriptome and chromatin accessibility data from iPSC-derived neurons with chromatin topology and genetic variants. Results We profile 3D chromatin conformation in neuronal progenitors (smNPCs) and mDANs using LowC, identifying changes in A/B compartments and topologically associated domains. PD SNPs are enriched near genes expressed in mDANs, and we predict 254 regulatory variants that create or disrupt TFBS. Using chromatin conformation data, we link variants to target genes. At the BAG3 and SCARB2 loci, reporter assays in mDANs show reduced transcription driven by PD-associated alleles. Knock-down of NR2C2, a putative SCARB2 regulator, increases SCARB2 expression in differentiating neurons. The PD-associated SCARB2 allele shows reduced chromatin accessibility in mDANs and is associated with decreased expression in brain eQTL data. Insertion of PD-associated BAG3 allele by prime editing reduces chromatin accessibility across cell types, consistent with altered binding of LIM-homeodomain transcription factors. Conclusions Together, these results prioritize functional PD SNPs and show that variants at SCARB2 and BAG3 modulate gene expression in mDANs, providing mechanistic insight into PD. Funding This work was supported by the Luxembourg National Research Fund (FNR), Fondation du Pélican de Mie et Pierre Hippert-Faber, Luxembourg Rotary Foundation, and the German Research Foundation (DFG).

↑ Beginning

# LAT1-driven metabolic–epigenetic reprogramming underlying radiobiological resilience in glioblastoma: implications for BNCT

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Glioblastoma (GBM) is an aggressive brain tumor characterized by recurrence, therapy resistance, and cellular plasticity, features closely linked to epigenetic dysregulation. Aberrant chromatin remodeling, DNA methylation, and histone modifications promote metabolic adaptation and stress-response programs that sustain GBM progression and treatment resistance. Boron Neutron Capture Therapy (BNCT) is a targeted radiotherapy based on selective boron-containing compounds accumulation in tumor cells followed by neutron irradiation, inducing localized cytotoxicity while sparing healthy tissue. BNCT efficacy depends on LAT1, a key amino acid transporter overexpressed in tumors that mediates boron uptake; however, its epigenetic role in GBM remains poorly defined. Methods A dual approach was used to investigate LAT1 functional and epigenetic relevance in GBM. LAT1 was genetically silenced in GBM cell lines, while its expression was pharmacologically modulated using HDACis. Transcriptomic profiling and pathway enrichment analyses assessed stress adaptation programs. Tumor growth was evaluated in 2D and 3D models. Metabolic profiling and immunoblotting assessed glycolytic activity and metabolism–epigenetic coupling. Boron uptake was quantified by mass spectrometry. Results LAT1 silencing significantly impaired proliferative capacity and tumor growth. Transcriptomic and metabolic analyses showed suppression of glycolytic programs and reduced glycolytic activity after LAT1 depletion. HKII and ACLY levels were reduced, indicating decreased glycolytic flux and lower cytosolic acetyl-CoA availability. Since ACLY-derived acetyl-CoA is critical for histone acetylation and chromatin remodeling, global histone acetylation was assessed as a marker of metabolic–epigenetic coupling. LAT1-silenced cells showed reduced total histone acetylation, supporting a role for LAT1 in epigenetic regulation in GBM. Functional assays revealed reduced glycolysis and impaired adaptation to energetic stress. Mass spectrometry demonstrated reduced boron uptake following LAT1 silencing. Conversely, HDAC inhibition increased LAT1 expression, suggesting that epidrug-mediated LAT1 upregulation may enhance boron accumulation and improve BNCT efficacy. Conclusions These findings support a dual epigenetic and metabolic role for LAT1 in GBM. LAT1 depletion disrupts metabolic programs required for tumor growth and stress adaptation, while its pharmacological upregulation through HDACis may optimize BNCT efficacy. Funding This work was supported by the NRRP PNC, the project ANTHEM – AdvaNced Technologies for Human-centrEd Medicine, project n. PNC0000003.

↑ Beginning

# Maternal mental health during pregnancy and placental DNA methylation

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background: Symptoms of anxiety and depression during pregnancy are common, affecting up to 20% of women in Europe. These conditions have been associated with adverse birth outcomes and an increased risk of neurodevelopmental problems in offspring, including cognitive, emotional, and behavioral difficulties. Emerging evidence suggests that prenatal maternal mental health may influence fetal development through alterations in placental epigenetics, particularly DNA methylation (DNAm). Studying these mechanisms may help identify the biological pathways linking maternal mental health to offspring development. Aim: We examined the association between prenatal maternal depression and anxiety symptoms and placental DNAm patterns. Methods: We analysed 458 mother–child pairs from the Barcelona Life Study Cohort (BiSC). Maternal mental health was assessed using the SCL-90-R, generating continuous and binary measures of anxiety, depression, and combined symptoms. Placental DNAm was measured using the Illumina Infinium MethylationEPIC array. Epigenome-wide association studies (EWAS) were conducted using robust linear models adjusted for relevant covariates. Additional analyses included sex-stratified EWAS and sensitivity models restricted to participants of European ancestry and to pregnancies without major complications or adverse birth outcomes. Results: Four CpGs reached false discovery rate (FDR) significance in the main analyses. Sex-stratified analyses identified 28 CpGs (11 in female placentas and 17 in male placentas), with partial evidence of sex-specific associations. Annotated genes were involved in energy metabolism, insulin signaling, SMAD2/3 signaling, and programmed cell death. Enrichment analyses highlighted pathways related to lipid homeostasis, Ras signaling, and nervous system and synapse functioning. Conclusions: This EWAS identifies novel sex-specific and sex-independent placental DNAm signatures associated with maternal depression and anxiety symptoms, supporting biologically plausible pathways linking maternal mental health to the intrauterine environment and fetal development. Ongoing efforts within the PACE consortium, including a placental meta-analysis, will further strengthen the robustness and generalizability of these findings.

↑ Beginning

# Oral Pathogens Induce Trained-Like Immunity in Gingival Fibroblasts: Epigenetic Perspective on Stromal Memory in Periodontitis

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

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**Abstract**:
Background Periodontitis is a chronic inflammatory disease driven by dysbiotic microbial communities. While trained immunity has been extensively described in myeloid cells, its presence and epigenetic regulation in tissue-resident stromal cells remain poorly understood. Gingival fibroblasts (GFs), the dominant cell population in periodontal tissue, actively participate in immune responses. Whether oral pathogens induce long-term epigenetic reprogramming in GFs is unclear. Methods Primary human GFs from multiple donors were infected with Porphyromonas gingivalis or Fusobacterium nucleatum. Following bacterial clearance and a recovery phase, cells were restimulated with TNF-α or bacteria. Cytokine production (ELISA), gene expression (qPCR, RNA-seq), NF-κB activation (Western blot), and DNA methylation (Reduced Representation Bisulfite Sequencing) were assessed. To explore epigenetic mechanisms, cells were treated with inhibitors targeting histone-modifying enzymes, including bromodomain, histone deacetylase (HDAC), and histone acetyltransferase (HAT) inhibitors. Results Pre-exposure to live bacteria induced a persistent, trained-like phenotype in GFs, characterized by enhanced production of IL-6 and MMP1 and MMP3, and sustained NF-κB activation upon secondary stimulation. This response persisted for at least 7 days and was independent of proliferation. Transcriptomic analysis revealed changes in genes associated with tissue remodeling and signaling pathways. Despite clear functional reprogramming, DNA methylation profiling revealed no significant differences between primed and control cells, suggesting that stable DNA methylation changes do not underlie this phenotype. In contrast, pharmacological targeting of histone-modifying enzymes produced variable and inconsistent effects. The impact of inhibitors differed markedly between donor-derived GF lines, with some showing attenuation of the trained response and others no effect or opposite trends, resulting in non-conclusive outcomes. Conclusions GFs exhibit trained-like inflammatory memory following bacterial exposure; however, this phenotype is not associated with detectable DNA methylation changes. The heterogeneous and donor-dependent effects of histone-modifying enzyme inhibition suggest a complex and context-dependent epigenetic regulation that cannot be generalized. These findings highlight stromal cell plasticity and indicate that non-canonical or transient epigenetic mechanisms may underlie inflammatory memory in periodontal disease. Funding This work was supported by the National Science Centre, Poland grant (2021/43/B/NZ5/03165) to AMG.

↑ Beginning

# Single-cell multiome analysis reveals AP-1–driven regulatory reprogramming during endocrine therapy in ER positive breast cancer

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Dr. Thomas Fleischer (Group Leader)

**Co-authors**:

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**Abstract**:
Endocrine therapy is central in ER positive breast cancer, yet acquired resistance remains a major cause of relapse. The regulatory mechanisms underlying adaptive tumor cell states during treatment, particularly at the level of enhancer-mediated transcription, are not well understood. We applied single-cell multiome profiling (scRNA-seq and scATAC-seq) to longitudinal tumor samples collected before, during and after neoadjuvant endocrine therapy. Using SCENIC+, we reconstructed gene regulatory networks across cell populations, identifying 43 high-confidence enhancer-driven regulons (eRegulons). Immune-related regulons mapped specifically to immune cells, while ESR1 (ER) activity dominated tumor cells at baseline, consistent with hormone-dependent transcription. Strikingly, longitudinal analysis revealed a dynamic shift in regulatory programs within epithelial tumor cells during treatment. In multiple patients, we observed increased activity of AP-1 transcription factors (FOS/JUN) on-treatment and post-treatment, accompanied by context-dependent activation of additional regulators including AR and GRHL2. These changes were not uniformly present at baseline but emerged or expanded under treatment, indicating a consistent shift toward therapy-associated regulatory programs. Together, this identifies AP-1-associated enhancer activity as a recurrent, treatment-emergent regulatory state, implicating stress-responsive transcriptional circuits as central features of endocrine resistance and candidate therapeutic targets.

↑ Beginning

# Transposable elements reorganise the 3D genome structure in CDK4/6 inhibitors resistant breast cancer

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Dr. Joanna Achinger-Kawecka (SAiGENCI, Adelaide University, Adelaide, South Australia, Australia)

**Abstract**:
Cyclin-dependent kinases 4 and 6 inhibitors (CDK4/6i) are a standard-of-care treatment in combination with endocrine therapy for hormone-receptor positive advanced breast cancer. Recent studies have revealed that activation of transposable elements (TEs), leading to viral mimicry response, is one of the key mechanisms by which CDK4/6i leads to tumour inhibition in breast cancer. However, acquired resistance to CDK4/6i is frequently observed and the potential role of TEs in its development is unclear. Here, we identified and characterized TEs that are robustly activated in patient-derived xenograft (PDX) breast cancer models of acquired CDK4/6i resistance. We found distinct TE subfamilies, namely endogenous retrovirus (ERV) long terminal repeats (LTRs), which are transcriptionally activated in CDK4/6i resistance both in vitro and in vivo with epigenetic reprogramming leading to increased chromatin accessibility, localized DNA demethylation, enrichment for active histone marks (H3K27ac) and co-occupation of key oncogenic transcription factors, including FOXA1, STAT1, p53 as well as architectural protein CTCF. Using chromosome conformation capture (Hi-C and Capture Hi-C) experiments, we show that regulatory ERVs contribute to 3D genome reorganisation in CDK4/6i resistant PDXs. We find a genome-wide increase in 3D genome compartment structure, with activated ERVs enriched at strong A-type compartments and new topologically associated domain (TAD) boundaries at TE-mediated CTCF binding. Importantly, activated regulatory ERVs contact and regulate expression of distal genes by creating new 3D chromatin interactions at transcription factor binding sites, indicating potential regulatory role in CDK4/6i resistance. Together, our results suggest that activated TEs contribute to oncogene overexpression by hijacking transcription factors to alter 3D chromatin structure in CDK4/6i resistant ER+ breast cancer.

↑ Beginning

# Uncovering the role of epigenetics in LACTBs tumor suppressive landscape

To be considered for an Oral Presentation | 3. Epigenetic mechanisms in human disease

**Main author**: Sr. Gabriel Miguel Rodriguez Gomez (Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences · Postdoctoral researcher)

**Co-authors**:

- Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences: Dra. Zuzana Kečkéšová (Group Leader)

**Abstract**:
The mitochondrial protein LACTB has been proposed to function as a tumor suppressor in breast cancer, yet the molecular mechanisms underlying its activity remain incompletely understood. To identify mediators of LACTB-dependent tumor suppression, we performed BioID proximity labeling and identified the mitochondrial one-carbon metabolism enzyme SHMT2 as a candidate interactor. SHMT2 is upregulated in breast cancer and correlates with poor patient prognosis. Direct interaction between LACTB and SHMT2 was validated by dot blot, proximity ligation assays and co-immunoprecipitation in breast cancer cell lines, demonstrating a specific mitochondrial interaction. Functionally, inducible LACTB overexpression reduced SHMT2 protein levels across multiple breast cancer cell lines without decreasing SHMT2 mRNA, indicating post-transcriptional regulation. A LACTB mutant defective in protein–protein interactions failed to bind or downregulate SHMT2, demonstrating that physical interaction is required. In vitro assays using recombinant proteins further showed that LACTB promotes SHMT2 degradation in an enzymatic activity dependent manner. Because SHMT2 catalyzes the conversion of serine to glycine to fuel one-carbon metabolism and S-adenosylmethionine (SAM) production, we investigated metabolic consequences of LACTB induction. Untargeted metabolomics and ^13C-serine isotope tracing revealed decreased glycine and SAM levels and reduced serine flux into one-carbon–derived metabolites, despite compensatory upregulation of serine biosynthesis enzymes. Consistent with reduced SAM availability, LACTB overexpression induced global DNA hypomethylation. Integrated BS-seq and RNA-seq analyses identified 119 genes that were both hypomethylated and transcriptionally upregulated upon LACTB induction. Among these were the tumor suppressor regulators as ZNF423, whose enhancer regions exhibited reduced methylation and active chromatin marks. Together, our findings uncover a mitochondrial epigenetic axis in which LACTB promotes SHMT2 degradation, reduces DNA methylation, and activates tumor suppressive transcriptional programs in breast cancer cells.

↑ Beginning

# A regulatory eRNA at the Nanog locus controls epigenetic stability and chromatin architecture in mouse embryonic stem cells

To be considered for an Oral Presentation | 4. Epigenetic and chromatin in stemness, aging and development

**Main author**: Dr. Mariella Cuomo (Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", 80131, Naples, Italy. · 1)

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**Abstract**:
Background Enhancers regulate gene expression through three-dimensional chromatin organization, yet the functional role of enhancer-derived RNAs (eRNAs) remains poorly understood. The Nanog locus in mouse embryonic stem cells (mESCs) is controlled by three active super-enhancers (-5 kb, -45 kb, and +60 kb relative to the transcription start site), all of which physically interact with the Nanog promoter but differ in their transcriptional impact. Results We identify and functionally characterize the eRNA transcribed from the -5 kb super-enhancer of Nanog, demonstrating that it is required to maintain an active epigenetic state at the locus. Loss of this eRNA triggers a progressive epigenetic reprogramming characterized by DNA methylation spreading from a putative initiating CpG site, extensive chromatin remodeling, and disruption of enhancer-promoter looping. These changes lead to stable silencing of Nanog and drive differentiation of mESCs toward an endodermal lineage. Notably, re-expression of Nanog alone fails to restore the original epigenetic configuration or chromatin architecture, indicating that the -5 kb eRNA acts upstream of transcriptional output and is required for locus integrity. Mechanistically, the -5 kb eRNA interacts with RAD21, suggesting a role in cohesin-mediated chromatin looping. In this model, eRNA expression precedes and enables Nanog transcription, acting as a structural and epigenetic regulator of pluripotency. Conclusions Our findings identify the -5 kb Nanog eRNA as a critical upstream regulator of chromatin architecture and epigenetic stability at the locus. This work supports a functional model in which eRNAs actively contribute to the maintenance of enhancer-promoter communication and pluripotency, with potential implications for developmental regulation and diseases involving aberrant Nanog expression.

↑ Beginning

# Comparison of a novel microRNA clock with established DNA methylation clocks in determining gestational age.

To be considered for an Oral Presentation | 4. Epigenetic and chromatin in stemness, aging and development

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**Abstract**:
Introduction: DNA methylation (DNAm) has shown promise as a biological marker of gestational age (GA), through epigenetic GA clocks. Here, we aimed to characterize how plasma circulating miRNAs – another mechanism involved in gene regulation – associate with gestational age at birth, and to construct a miRNA-based GA clock (miRClock-GA), examining its relation to DNAm-based clocks and predictive value for a range of developmental outcomes beyond clinically-assessed GA. Methods: We leveraged 2083 umbilical cord plasma-derived circulating miRNAs from 1695 participants from the Generation R Study. First, we performed linear regressions to identify miRNome-wide significant miRNAs associated with gestational age. Second, we applied elastic net regression to construct a miRClock-GA. These steps were validated in 213 samples from an indipendent cohort, Gen3G. Finally, we computed a measure of age acceleration (miRClock-AA) and evaluated association of miRClock-GA and miRClock-AA with child developmental outcomes up to 17 years of age, including comparisons with methylation-based DNAmClocks. Findings: We identified 123 miRNAs associated with GA, with miR-150-5p showing the strongest positive association (B = 0.244, SE = .036, P = 2.3e-11) and miR-373-3p the strongest negative association ( B = -0.255, SE = .065, P = 8.6e-5). MiRClock-GA correlated consistently with GA in Generation R train (r_range = 0.62 - 0.72) and test sets (r_range = 0.45 - 0.52) as well as in the independent validation cohort (r_Gen3G = 0.33). Correlations between miRClock-GA and DNAmClocks varied from weak to moderate (r_range = 0.28 - 0.42). MiRClock-AA explained significant variance in birthweight and childhood BMI beyond clinical GA, when controlling for an extended set of maternal covariates. Conclusions: This study reveals widespread associations between circulating miRNAs and GA, supports the use of miRClock-GA as a consistent, well-performing biological marker of GA, with miRClock-AA predicting birthweight and childhood BMI beyond GA itself. Our findings provide a broader perspective on the potential utility of miRNAs as early markers of (altered) development. Funding: This research was supported by the European Union's Horizon Europe Research and Innovation Programme (FAMILY, No.101057529) and by the European Research Council (TEMPO, No.101039672).

↑ Beginning

# EpiDirect®-Clock: a rapid, cost-efficient, bisulfite-free, qPCR-based age prediction tool for forensic casework

To be considered for an Oral Presentation

4. Epigenetic and chromatin in stemness, aging and development

**Main author**: Sra. Maria Mindegaard (R&D Scientist)

**Abstract**:
Background DNA methylation at specific CpG sites is often used for chronological age estimation. Most methodologies require bisulfite conversion to preserve the methylation signature during PCR amplification; however, bisulfite conversion is a harsh chemical-based process, which causes substantial DNA fragmentation and loss. High DNA amounts (>50 ng) are required for a successful conversion, making the current methodologies incompatible with many forensic trace samples, where DNA is often limited. Method In this study, a novel methodology, the EpiDirect®-Clock, was developed and tested. The EpiDirect®-Clock is a qPCR assay that enables direct methylation estimation without any sample pretreatment. The assay relies on intercalating nucleic acid primers, which have different affinities for DNA sequences, depending on the methylation status. The assay targets seven CpG sites in the ELOVL2 gene and quantifies the overall methylation level. Results DNA purified from 150 blood samples was diluted to 1 ng/µL (DNA input 5 ng) and analysed on a QuantStudio5 (Thermo Fisher Scientific). Ct values were used to develop and validate an age prediction model. The selected model (a third-degree polynomial) was validated on 60 samples. The model's performance on the training and test datasets was measured as a mean absolute error (MAE) of 4.47 years (root mean squared error (RMSE) of 5.95 years, R2 = 0.83) and 4.73 years (RMSE of 6.27 years, R2 = 0.81), respectively. These results are comparable with or even outperform other age prediction models currently used. Most importantly, a preliminary sensitivity analysis showed that the DNA input could be lowered to 500 pg without compromising model efficiency substantially. This represents a 100-fold reduction in DNA input compared to bisulfite-based methods (>50 ng), making the EpiDirect®-Clock compatible with trace forensic samples. Conclusion The EpiDirect®-Clock provides a rapid, low input, cost-efficient, and bisulfite-free method for age estimation, with significant advantages for forensic casework. The limited biological material needed combined with a simple protocol for instrumentation already present in most forensic genetic laboratories make this approach highly appealing and may replace more expensive and sample-consuming methods such as sequencing or microarray technology.

↑ Beginning

# Fueling the Nucleus: Nuclear GDH1 Links Metabolism and Epigenetic Regulation to Stem Cell Identity.

To be considered for an Oral Presentation

4. Epigenetic and chromatin in stemness, aging and development

**Main author**: Dr. Roxane Verdikt (KULeuven)

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- Baylor College of Medicine: Prof. Kian Peng Koh
- Institut Curie: Prof. Reini F. Luco

**Abstract**:
How metabolic activity is wired into chromatin regulation during stem cell fate transitions remains a central question in developmental epigenetics. Here, we show that glutamate dehydrogenase 1 (GDH1), an enzyme that generates alpha-ketoglutarate (aKG) from glutamate, localizes to the nucleus of mouse embryonic stem cells, where it supports pluripotent identity. GDH1 depletion alters stemness-associated transcription factor networks in both naïve and primed pluripotent states, indicating a broad role in cell state control. We further find that nuclear GDH1 is enzymatically active and that its metabolic activity underpins its control of gene expression. Mechanistically, GDH1 interacts with two major nuclear regulatory layers: it engages the splicing machinery and influences TET proteins, thereby linking local metabolism to RNA processing and chromatin modification. These results uncover a nuclear metabolic node that coordinates epigenetic and co-transcriptional regulation during pluripotent stem cell transitions.

↑ Beginning

# “Writing and Erasing the Chromatin Code: KMT5a and p53 Cooperate to Control p62/SQSTM1 in Glioblastoma”

To be considered for an Oral Presentation | 5. Epigenetics in drug discovery and therapeutics

**Main author**: Dr. Rosa Della Monica (1)

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**Abstract**:
Background Epigenetic regulation of transcription is a key driver of gene expression in cancer, largely through histone modifications that control chromatin accessibility. KMT5a, a histone methyltransferase responsible for H4K20 mono-methylation, is overexpressed in glioblastoma and linked to poor prognosis. Its role in transcriptional control within a broader epigenetic code remains unclear. Here, we investigated how KMT5a regulates transcription of p62/SQSTM1 in relation to chromatin state and p53 activity. Results KMT5a inhibition or silencing led to strong upregulation of p62/SQSTM1 in glioblastoma cells. KMT5a directly binds the p62 promoter, depositing the repressive H4K20me1 mark and promoting chromatin compaction. This repression is reinforced by hierarchical methylation via KMT5b and KMT5c, while PHF8 counteracts this state by demethylation. p62/SQSTM1 activation upon KMT5a loss occurred only in p53-deficient cells, whereas wild-type p53 restored transcriptional repression. Under metabolic stress, induction of p62 required both removal of H4K20 methylation and loss of p53 occupancy, indicating coordinated epigenetic and transcription factor control. Conclusions KMT5a drives transcriptional repression of p62/SQSTM1 through a hierarchical H4K20 methylation program integrated with p53 status. This defines a coordinated epigenetic–transcriptional axis in glioblastoma and highlights histone methylation as a dynamic regulator of gene expression with potential therapeutic relevance.

↑ Beginning

# CDK12/CDK13 inhibition disrupts transcriptional elongation and replication fork progression in glioblastoma

To be considered for an Oral Presentation | 5. Epigenetics in drug discovery and therapeutics

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**Abstract**:
Glioblastomas are the most prevalent and aggressive malignant brain tumors, characterized by hypertranscription and dependence on neurodevelopmental transcription factors. The transcriptional cycle is regulated by phosphorylation of the C-terminal domain (CTD) of RNA polymerase II (RNAPII) by transcriptional cyclin-dependent kinases (tCDK), including CDK7, CDK9, CDK12, and CDK13. Here we find that glioblastoma stem cells (GSC) are selectively sensitive to CDK12/CDK13 inhibition, whereas CDK7 and CDK9 inhibition cause non-specific cytotoxicity. This selective targeting halts GSC and organoid proliferation, curtails GSC invasion and suppresses tumor growth in a xenograft mouse model. In GSCs, CDK12/CDK13 inhibition leads to a rapid and genome-wide loss of serine-2 phosphorylation (pSer2) of the RNAPII CTD, abolishing transcriptional elongation and a transcriptional program sustained by key neurodevelopmental transcription factors. CDK12/CDK13 inhibition unexpectedly arrests DNA replication and fork progression in a manner distinct from the effect of inhibiting other tCDKs. This dramatic arrest precedes DNA damage response activation and cell cycle arrest, directly linking RNAPII elongation to fork dynamics and revealing a previously unrecognized dependence of DNA replication on CDK12/CDK13-RNAPII regulation.

↑ Beginning

# DEVELOPMENT OF CHEMICAL PROBES FOR METHYL-LYSINE READER DOMAINS IN EPIGENETIC REGULATION

To be considered for an Oral Presentation | 5. Epigenetics in drug discovery and therapeutics

**Main author**: Dr. Filomena Saulino (Newcastle University · Research associate)

**Co-authors**:

- Newcastle University: Prof. Akane Kawamura (Principal investigator)

**Abstract**:
Background: Epigenetic mechanisms regulate gene activity without altering the DNA sequence, influencing key biological processes including transcription, cell cycle progression, and DNA damage repair. 1 Lysine methylation is one of these mechanisms, coordinated by writers, erasers, and readers, which respectively introduce, remove, and interpret epigenetic marks.2 Plant Homeodomain (PHD) fingers are zinc-coordinating reader domains found in over 100 human proteins that recognise modified and unmodified lysine residues on the histone H3 N-terminal tail.3 While broadly classified by their recognition of H3K4 methylation status, PHD fingers can display diverse recognition patterns for combinatorial post-translational modification (PTMs). 4,5 Dysregulation of PHD finger-containing proteins is implicated in cancer, neurological, and autoimmune diseases, making them compelling targets. 6-9 Despite their disease relevance, PHD fingers remain largely undruggable due to shallow binding pockets and current probessuffer from poor potency and selectivity.10,11 Cyclic peptides (CPs) represent a promising strategy, offering greater conformational flexibility than small molecules to engage challenging protein surfaces. 12 In 2022, we reported OC9, the first CP targeting the KDM7 subfamily PHD finger with nanomolar affinity, though it lacked intra-subfamily selectivity.13 This work aims to elucidate PHD finger PTM recognition biology and develop novel, selective cyclic peptides as chemical probes for new therapeutic strategies in cancer. Methods, Results and conclusion: To explore the molecular mechanisms of combinatorial PTM recognition by KDM7 PHD fingers, an AlphaScreen binding assay was developed against a library of modified histone H3 peptides. In contrast to KDM7A PHD, KDM7B and KDM7C PHD fingers were found to recognise R2 dimethylation in the presence of K4me3, suggesting a divergent R2 binding pocket architecture among KDM7 family members and pointing to previously unappreciated functional differences within the subfamily. Building on these findings, competitive mRNA display was employed to identify cyclic peptides selectively targeting the PHD domain of KDM7B. The binding profiles of selected CPs were characterised using biophysical assays, including AlphaScreen, biolayer interferometry, and DSF. These results provide new insights into the molecular basis of PHD finger PTM recognition and establish a foundation for the rational development of selective chemical probes targeting this challenging yet therapeutically relevant protein family. Funding: This project is funded by the European Research Council (ERC).

↑ Beginning

# HEPATOCYTE-SPECIFIC DNMT3A AND DNMT3B KNOCKOUT MICE IS LESS SENSITIVE ACETAMINOPHEN THAN WILD-TYPE

To be considered for an Oral Presentation

5. Epigenetics in drug discovery and therapeutics

**Main author**: Dr. Tamas Aranyi (1Department of Molecular Biology, Semmelweis University, Budapest, Hungary)

**Abstract**:
NA (hydroxy)methylation play major role in differentiation and maturation in various tissues. Our aim in this study was to characterize the phenotype of mice after liver-specific elimination of the de novo DNA methylation apparatus. Mouse strains carrying Dnmt3aFlox/Flox/3bFlox/Flox and Albumin-Cre (Alb-Cre) or Alfp-Cre transgene were generated to abolish the expression of both de novo DNMTs(DKO) specifically in hepatocytes at different phases of embryogenesis. Mice were viable without apparent phenotype. We investigated DNA methylation, gene expression, metabolic activity, and liver histology. In addition, we performed hepatotoxicity tests. In Alb-Cre mice, we observed significant DNA hypomethylation at base resolution, while no global methylation loss was detected. The hypomethylation was generally located in the distal promoter regions. Only minor gene expression differences were present between DKO and wild-type (WT) animals at 3 weeks. DNA methylation and gene expression patterns significantly changed during maturation and became prominent between 40 weeks and 3 weeks old young mice. At 40 weeks, mild but significant differences were observed between WT and DKO mice as well. To test whether hepatotoxicity experiments affect differentially the DKO and WT mice, we performed 48h CCl4 treatments. These experiments showed a very intense response in the acute phase, with only a few genotype-dependent gene expression difference. We then focused on the Alfp-cre mice, and investigated the effect of acetaminophen (APAP), a clinically relevant molecule also often used to test liver toxicity. Here, we observed that significant gene expression differences occur upon the treatment, both at 6h and at 24h after the treatment. We observed, that the WT mice underwent a stronger metabolic gene expression downregulation and a more intense endoplasmic reticulum stress gene expression upregulation. In addition, the xenobiotic metabolism pathway was more strongly upregulated in the DKO mice, suggesting an earlier and more efficient reaction to the toxic agent in these animals. At 24h after the treatment, the gene expression remained still highly perturbed (~4000 genes differentially expressed relative to the control), but no more genotype-dependent difference could be observed. In conclusion, our results show that de novo DNA methyltransferases participate in the reaction to hepatotoxic stress.

↑ Beginning

# Modulation of histone code to manipulate macrophage responses to P. gingivalis

To be considered for an Oral Presentation | 5. Epigenetics in drug discovery and therapeutics

**Main author**: Sra. Dominika Materniak (Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland · PhD Candidate)

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- Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland: Dr. Aleksander Grabiec (Associate Professor)

**Abstract**:
The submission system prevents full abstract submission due to differences in word-counting methods compared to Microsoft Word (which indicates 334 words); the complete abstract is available in the PDF. Background Macrophages are a highly versatile cell population that can adopt a variety of functional states in response to external stimuli. Such transitions are only possible due to a lasting mark on their epigenetic landscape. Inflammatory cues, particularly those arising from bacterial infection, are key drivers of this process. This study employed Porphyromonas gingivalis (Pg), a central pathogen in periodontitis, to examine alterations in the histone code and to investigate strategies for its modulation aimed at mitigating macrophage-mediated inflammation. Methods Monocyte-derived macrophages were generated by culturing human monocytes in complete RPMI medium supplemented with GM-CSF. To assess alterations in H3K27ac and H3K4me3 following 4 h infection with P. gingivalis, CUT&RUN-LoV-U (Cleavage Under Targets and Release Using Nuclease—Low Volume Urea) was performed. In addition, the expression of key histone-modifying enzymes was evaluated. The histone landscape was further modulated by 24 h pretreatment with histone deacetylase inhibitors (HDACi), histone acetyltransferase inhibitors (HATi), and BET protein inhibitors (BETi), followed by evaluation of downstream effects, including transcriptomic changes, reactive oxygen species production, bacterial internalization, and cytokine secretion. Results A global decrease in H3K27ac levels was observed following infection with P. gingivalis, accompanied by altered H3K4me3 profiles at key inflammatory loci, including TNF, IL6, and IL1A. Elevated H3K4me3 levels were consistent with RNA-seq–based upregulation of TNF, IL1A, IL1B, and PTGS2. In addition, class I HDACs and JMJD3/6 were upregulated. Pharmacological inhibition using HDACi, HATi, and BETi attenuated Pg-induced inflammation, as evidenced by reduced production of proinflammatory cytokines (IL6, IL8, TNF, CCL2, CCL5) and decreased bacterial internalization. Notably, only HDAC inhibition led to increased reactive oxygen species generation. RNA-seq analysis further revealed distinct mechanisms of action of the inhibitors, involving regulation of cell cycle, cellular metabolism, and chromatin remodeling.

↑ Beginning

# Optimizing T Cell Expansion: Mitigating Culture-Associated DNA Methylation Changes with Decitabine and Vitamin C

To be considered for an Oral Presentation | 5. Epigenetics in drug discovery and therapeutics

**Main author**: Erik Meyer (Institute for Stem Cell Biology, Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany)

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**Abstract**:
The production of T cell therapeutics, such as chimeric antigen receptor (CAR) T cells, requires in vitro culture. However, this entails changes in the DNA methylation landscape which can impair T cell function. We previously demonstrated that culture-associated DNA hypermethylation at specific CpGs was associated with reduced overall-survival in CAR T cell therapy. We therefore aim for a better understanding of what drives culture-associated DNA methylation changes and how this might be counteracted. Primary T cells were cultured for 21 days with or without CD3/CD28 agonist TransAct. Only activated T cells exhibited significant proliferation and substantial changes in DNA methylation patterns, whereas non-activated T cells remain largely unchanged. Furthermore, when we activated CAR T cells by an in vitro killing assay we observed again that the culture-associated DNA methylation patterns increased. In contrast, we did not see similar changes in data of CAR T cells post-infusion, indicating that the changes are primarily due to in vitro expansion. Subsequently, we explored if the effect could be mitigated by the demethylating agent decitabine (DAC). DAC led to significant hypomethylation and mitigated culture-associated DNA methylation, while the effects on proliferation and immunophenotype were rather moderate. We then tested vitamin C supplementation, which is a cofactor for TET2. In fact, vitamin C treatment also resulted in significant hypomethylation, less culture associated DNA methylation changes, and a significant increase in proliferation and preservation of CD4/CD8 ratio. To enable better tracking of culture-associated DNA methylation for quality control and optimization of culture conditions, we developed a three-CpG epigenetic predictor based on digital PCR. Our results demonstrate that DNA methylation changes that arise in T cells during culture are linked to in vitro proliferation following T cell activation. This effect can be counteracted by DAC or vitamin C, which may better preserve T cell function during expansion.

↑ Beginning

# Targeted epigenetic interventions enable next-generation T cell products for adoptive cell therapies.

To be considered for an Oral Presentation | 5. Epigenetics in drug discovery and therapeutics

**Main author**: Prof. Julia Polansky (group lead)

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- Berlin Institute of Health at Charité Universitätsmedizin Berlin: Ramonique Lim; Dania Hamo; Marcel Finke; Frederik Hamm; Mingxing Yang; Kressler Christopher

**Abstract**:
Background – Epigenetic mechanisms are key regulators of T lymphocyte differentiation and function, and thus, also determine their efficacy as 'living drugs' in adoptive cell therapies. Therefore, leveraging innovative strategies for the targeted editing of epigenetic structures enable the development of functionally-improved, next-generation T cell products tailored for diverse clinical applications. Methods – We performed genome-wide DNA methylation profiling of primary human T cells spanning all thymic developmental stages as well as mature peripheral T cell subsets to identify epigenetic elements and mechanisms imprinting T cell phenotypes and functions. We then employed pharmacologic and CRISPR dCas9-mediated epigenetic editing approaches for targeted epigenetic interventions, to generate desired functional T cell phenotypes for adoptive cell therapy. Results – We identified key epigenetic regulatory elements, such as promoters and enhancers, as critical determinants of T cell subset identity. Using these elements as targets, we employed CRISPR-dCas9-based epigenetic editing to successfully reprogram pro-inflammatory T cells towards an anti-inflammatory regulatory T cell (Treg) phenotype. This approach allowed us the de novo generation of improved therapeutic Treg products from multiple cellular sources, overcoming current challenges of Treg product manufacturing. Additionally, we characterized the mechanisms that erode cell type-specific epigenetic structures during strong proliferation episodes such as during cell product manufacturing. These data allow us to develop targeted approaches for the epigenomic stabilization of highly expanded cell products for therapeutic applications. Finally, insights on the epigenetic remodeling taking place during thymic T cell development enabled us to validate an iPSC-based T cell generation protocol for its utility as an T cell production platform for cell products. Conclusions – Our findings underscore the transformative potential of epigenomic profiling and targeted epigenetic interventions in advancing adoptive T cell therapies, which overcome current manufacturing challenges and support the future development of off-the-shelf therapeutic products.

↑ Beginning

# Treatment with epigenetic inhibitors restores cGAS–STING signalling in immunologically “cold” tumors

To be considered for an Oral Presentation | 5. Epigenetics in drug discovery and therapeutics

**Main author**: Dra. Camilla Manfredi (Department of Molecular Medicine, University of Rome La Sapienza, 00185, Rome, Italy · 1)

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**Abstract**:
Background Conventional immunotherapies, show limited efficacy in “cold” adult and pediatric tumors such as colorectal (CRC), prostate (PCa), lung cancer (LC) and neuroblastoma (NB), the most common extracranial solid tumor in childhood. cGAS-STING pathway, a key mediator of cytosolic DNA sensing,plays a crucial role in evoking an antitumor immune response by inducing type I-IFN release and potentially converting “cold” into “hot” tumors. However, this pathway is frequently silenced in tumors due to epigenetic aberrations such as hypermethylation and reduced acetylation of cGAS/STING promoters. Methods cGAS/STING expression was assessed by scRNA-seq analysis in various “cold” tumors. TCGA and ENCODE were used to evaluate DNA methylation and histone acetylation levels at cGAS/STING promoters. Protein expression and pathway activation were evaluated by WB and qPCR. Type I-IFN release was quantified using the HEK-Blue SEAP reporter assay. Results In cell lines derived from “cold” adult and pediatric tumors, cGAS is expressed at very low levels and STING pathway is impaired as confirmed by scRNA-seq data in CRC, PCa, and LC tumor epithelial cells. TCGA and ENCODE analyses revealed increased DNA methylation levels along with reduced histone acetylation at cGAS and/or STING promoters. Accordingly, DNMT1 and DNMT3A expression levels were upregulated in “cold” tumor cell lines and in primary tumors compared with healthy subjects. A 20 compounds epidrug screening, targeting DNMT1,3A,3B, LSD1 and HDAC1,2,3,6, was performed to identify more specific and less toxic compounds targeting aberrant levels of DNA methylation and favouring local acetylation. A specific DNMT inhibitor significantly induced cGAS/STING expression, while the addition of HDAC inhibitor displayed synergistic effect on cGAS/STING re-expression in CRC, PCa, LC and NB cells. Stimulation with STING agonist, diABZI, enhances type I-IFN signalling and STAT1 phosphorylation, confirming pathway functionality in both adult and pediatric tumor models. Type I-IFN release detected by HEK-Blue cells, confirms type I-IFN secretion in “cold” tumor models, supporting STING pathway reactivation. Conclusions Coordinated DNMT and HDAC inhibition restores cGAS–STING expression in “cold” pediatric and adult tumors and reactivates type I-IFN signalling.

↑ Beginning

# Beyond the CpG: Dissecting the functions of atypical DNA methylation during early vertebrate development

To be considered for an Oral Presentation | 6. Epigenetics of the tree of life

**Main author**: Dr. Ozren Bogdanovic (Centro Andaluz de Biología del Desarrollo, CSIC-Universidad Pablo de Olavide-Junta de Andalucía, Seville, Spain · author order: 6)

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**Abstract**:
Cytosine DNA methylation is widespread in animal genomes and occurs predominantly at CG dinucleotides (mCG). While the roles of mCG, such as in genomic imprinting and genome stability, are well established, non-CG DNA methylation (mCH) remains poorly understood. In most vertebrate tissues, roughly 80% of CGs are methylated, whereas mCH levels are generally low. mCH is most prevalent in neural tissue, oocytes, and embryonic stem cells, and has been linked to neurodevelopmental disorders. Using CRISPR/Cas9 genome editing in zebrafish, we are functionally interrogating the contribution of mCH and its writer, Dnmt3ba, to early development in order to define the developmental requirements for proper mCH patterning. Building on these findings, we have generated stable dnmt3ba knockout lines which, in zebrafish, display redundancy in the maintenance of mCG patterns. These unique genetic contexts enable us to selectively perturb mCH deposition without globally disrupting canonical CG methylation. Strikingly, dnmt3ba knockouts exhibit widespread transcriptional dysregulation, significantly reduced nuclear size, and defects in the execution of zygotic genome activation (ZGA), suggestive of a critical role for mCH in coordinating early embryonic transcriptional programs. In addition, we observe a strong anticorrelation between mCH and the repressive histone mark H3K9me3, pointing to a potential complementary and stage-specific relationship between these epigenetic mechanisms during early development. Together, these findings support a model in which mCH represents a distinct and developmentally essential epigenetic layer that contributes to embryonic genome regulation independently of canonical CG methylation.

↑ Beginning

# Biomarker identification using DNA methylation signatures of patient-derived colorectal cancer tumoroids

To be considered for an Oral Presentation | 7. Epigenetic biomarkers

**Main author**: Prof. Gerda Egger (Medical University of Vienna · PI)

**Co-authors**:

- Medical University of Vienna: Sra. Loan Tran (PhD student); Sra. Kristina Draganic (PhD student); Dr. Raheleh Sheibani-Tezerji (Postdoc)

**Abstract**:
Patient-derived organoids (PDO) and tumoroids (PDTO) have gained considerable interest as innovative preclinical models for translational research because they closely recapitulate the molecular and phenotypic characteristics of tumors. These models preserve tumor heterogeneity and are well suited to predict patient responses to various treatments, including chemotherapy. To investigate the epigenetic stability of PDOs and PDTOs, we generated a collection of organoids from primary and metastatic colorectal cancer (CRC) tissues and matched normal adjacent mucosa. Genome-wide DNA methylation analyses revealed patient-specific DNA methylation subtypes, including PDTOs with the CpG island methylator phenotype (CIMP) and microsatellite instability (MSI). Furthermore, DNA methylation signatures, were well preserved between PDOs/PDTOs and their tissue of origin and remained remarkably stable during long-term cultivation. Strikingly, we identified a tumor-specific DNA methylation signature consisting of 39 hypermethylated CpG sites that was independent of CIMP subclass and anatomical site. These sites were unmethylated in normal colon tissues and PDOs but highly methylated in PDTOs and CRC specimens. Notably, the signature was already detectable in a subgroup of aggressive pre-malignant adenomas and showed a gradual increase in methylation levels during tumor progression. This DNA methylation signature represents a promising prognostic biomarker panel for early CRC diagnosis. We are currently investigating, whether the early detected changes in DNA methylation are also causally involved in tumorigenesis. Moreover, PDTOs showed differential sensitivities to DNA methylation inhibitors including decitabine and GSK3685032, highlighting the potential of epigenetic therapy for a subset of CRCs. In summary, PDTOs represent an advanced model for studying the role of the epigenome, particularly DNA methylation, and its impact on tumor progression and susceptibility to epigenetic inhibitors.

↑ Beginning

# DNA methylation signatures of human adaptation to chronic hypobaric hypoxia

To be considered for an Oral Presentation | 7. Epigenetic biomarkers

**Main author**: Dr. Davide Sacco (Department of Brain and Behavioral Sciences, Università di Pavia, 27100 Pavia, Italy · PhD)

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**Abstract**:
Hypobaric hypoxia is a condition of chronic exposure to reduced oxygen availability that triggers adaptive responses, which may become maladaptive if sustained. Andean high-altitude populations represent one of the best-adapted groups, characterized by a distinctive clinical profile not commonly observed in other populations, including increased hemoglobin and hematocrit and lower blood pressure. While genetic ancestry contributes to these adaptations, environmentally responsive mechanisms such as DNA methylation may also play a role, although their contribution remains incompletely characterized. To investigate this, we conducted a multilevel analysis of DNA methylation in whole blood from 96 Andean highlanders (Cerro de Pasco, 4,360 m; age 46 ± 15, 50% female) and 96 lowlanders (Lima, 150 m; age 47 ± 16, 50% female), profiling 866,000 CpG sites using Illumina EPIC v1 arrays. Differentially methylated positions (DMPs) and regions (DMRs) were identified through differential methylation analysis. High-dimensional mediation analyses assessed whether DNA methylation mediates the relationship between hypoxia exposure and several cardiovascular, hematological, and anthropometric phenotypes. Global methylation patterns, including epigenetic ageing and epigenetic drift, were also evaluated. All statistical models were adjusted for environmental, socio-behavioral, cellular, and genetic confounders, with genetic background derived from Illumina Global Screening Array v4 data. Findings were compared with an independent GEO dataset (N = 77; 41 highlanders, 36 lowlanders) using meta-analytic approaches. We identified 342 DMPs and 45 DMRs (FDR 0.05), enriched in pathways related to hypoxia response, metabolism, and cardiovascular functions, including angiogenesis. Mediation analysis identified specific CpG sites significantly mediating increases in hemoglobin and hematocrit, and reductions in oxygen saturation, hypertension risk, and waist circumference, with mediation contributions ranging from 3% to 21%. Epigenetic clocks showed a significant 3-year increase in epigenetic ageing in highlanders, without phenotypic associations, while epigenetic drift did not differ. Findings were consistent in meta-analytic comparisons. These findings highlight novel genes linked to pathways relevant to chronic hypoxia and their association with clinically relevant phenotypes. The increase in epigenetic ageing, in the absence of drift changes and phenotypic associations, suggests a potential adaptive rather than purely pathological process, providing new insights into human adaptation to chronic hypoxia and its physiological consequences.

↑ Beginning

# Epigenomic signatures during disease evolution in Preclinical Systemic Sclerosis

To be considered for an Oral Presentation | 7. Epigenetic biomarkers

**Main author**: Dr. Davide Barbuto (Department of Clinical Sciences and Community Health, Dipartimento di Eccellenza 2023-2027, University of Milan, 20122 Milan, Italy. · 1)

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**Abstract**:
Background: Systemic sclerosis (SSc) is a rare autoimmune disease characterized by fibrosis, vasculopathy and immune dysregulation. Disease development may begin years before overt manifestations, during a preclinical phase (PreSSc) marked by Raynaud's phenomenon, some SSc-specific autoantibodies and abnormal nailfold videocapillaroscopy (NVC), without fulfilling classification criteria. Approximately 50% of individuals with PreSSc progress to definite SSc within five years. Growing evidence suggests that epigenetic mechanisms, particularly DNA methylation changes, contribute to disease progression by modulating immune, vascular and fibrotic pathways and may represent biomarkers of disease trajectory. Methods: To date, 44 individuals with PreSSc have been enrolled at the Scleroderma Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan. PBMCs were collected at baseline (T0) and follow-up (T1, 6–12 months). Genomic DNA was extracted and methylome profiling performed using the Illumina EPIC BeadChip platform. Longitudinal analyses are ongoing to identify DNA methylation signatures associated with disease progression. Preliminary Results: The cohort includes 44 PreSSc subjects (40 women, 91%; 4 men, 9%), with a mean age of 48.6 ± 12.4 years and a median Raynaud's duration of 5.5 years (IQR 2–11). At baseline, 45% showed an early NVC pattern, 30% an active pattern and 25% a late pattern; anti-centromere antibodies were present in 30 patients (68%), anti-Scl70 in 10 (23%) and mixed positivity in 4 (9%). During follow-up, 8/44 subjects progressed to definite SSc, while the remainder remained in PreSSc, suggesting heterogeneous disease trajectories. Ongoing analyses compare methylation profiles between T0 and T1 and between individuals who progressed to definite systemic sclerosis vs those who did. Conclusions: This study aims to identify differentially methylated regions associated with progression from PreSSc to definite SSc, providing insight into early molecular determinants of disease evolution and potential stratification biomarkers.

↑ Beginning

# Lost in translation: How CpG site selection and assay design determine the clinical value of DNA methylation markers

To be considered for an Oral Presentation | 7. Epigenetic biomarkers

**Main author**: Dr. Louis Coussement (Department of Mathematical Modelling, Statistics and Bioinformatics, Ghent University, Ghent, Belgium · Post-doctoral researcher)

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- Department of Mathematical Modelling, Statistics and Bioinformatics, Ghent University, Ghent, Belgium: Prof. Wim Van Criekinge (Professor); Prof. Tim De Meyer (Professor)

**Abstract**:
Background: Despite the potential of DNA methylation as a biomarker in oncology, only about 20 of the more than thousands published DNA methylation biomarkers have been translated into clinical use. Previously, identification of the clinically most relevant region for biomarker genes has been determined as one of the major obstacles. These so-called “core regions”, are typically identified based on DNA methylation array data, which covers less than 5% of CpGs within the human genome. A similar limitation applies to other widely used techniques to measure DNA methylation in the clinic, such as methylation-specific PCR (MSP) on bisulfite converted DNA. Methods: Performing targeted bisulfite sequencing, we profiled DNA methylation at single CpG resolution in multiple amplicons for three known biomarkers: NDRG4 in colorectal cancer (CRC), GREM1 in clear-cell renal cell carcinoma (ccRCC) and LY75 in melanoma. DNA methylation degrees were assessed and correlated to clinical features. Diagnostic (NDRG4) and prognostic value (GREM1, LY75) for CpG methylation values were compared against a baseline model as well as between CpGs. Finally, we suggest a methylation sorted allele (MESA) plot for visualization of read-based information. Results: Amplicons around original biomarker MSP assays show a good diagnostic and prognostic value, confirming genomic location as an important feature in DNA methylation biomarker design. With a higher resolution, high inter- and intra-amplicon variability in diagnostic and prognostic performance was observed, indicating that not all CpGs contribute equally. Moreover, methylation signal clinical value was affected by primer design, particularly when CpGs were located within primers. Finally, MESA plots lead to insights surpassing those derived from summary statistics. Conclusions: The exact genomic location and assay design are critical determinants of DNA methylation marker performance. These findings underscore the need for single-base resolution information and careful assay design to unlock the full potential of DNA methylation markers in cancer diagnostics. Furthermore, these insights support a shift toward more refined DNA methylation marker assays, e.g. PCR-free PacBio or Oxford Nanopore single molecule sequencing to overcome key barriers in translating epigenetic biomarkers into clinical practice. Without this increased precision, promising biomarkers risk being lost in translation.

↑ Beginning

# Plasma histone monomers as novel diagnostic markers in adult glioblastoma

To be considered for an Oral Presentation | 7. Epigenetic biomarkers

**Main author**: Prof. Manlio Vinciguerra (Medical University Varna (Varna, Bulgaria); LUM University (Casamassima, Italy) · 6)

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- Medical University Varna (Varna, Bulgaria): Dra. Stefani Mariyanova Todorova (3); Prof. Yavor Enchev (4); Sra. Desislava Tsoneva (1)

**Abstract**:
Background: Glioblastoma (GB), the most aggressive primary brain tumor in adults, has a median survival of 12-18 months and lacks reliable minimally invasive biomarkers. Traditional diagnostics are limited by invasiveness and the inability to capture dynamic tumor changes. Circulating histones, reflecting tumor burden and epigenetic alterations, have emerged as potential liquid biopsy markers, but studies in adult GB are scarce compared to pediatric gliomas. The aim of this study was to characterize the plasma histone profile in adult GB patients versus healthy controls and evaluate its diagnostic potential. Methods: Plasma samples from 44 adult GB patients and 30 age-matched healthy controls were analyzed. Plasma levels of histones H3.1 and H3.3 were quantified by ELISA. Individual histones (H2A, H2B, H3, H4, macroH2A1.1, macroH2A1.2) and histone complexes (dimers/tetramers) were measured using an established imaging flow cytometry (ImageStreamX) multichannel detection approach. Results: GB patients exhibited significantly elevated plasma levels of individual histone monomers H2A, H3, macroH2A1.1, and macroH2A1.2, as well as markedly reduced H4 levels, compared to controls. Levels of histones H3.1- and H3.3 detected in plasma, showed no significant differences. MacroH2A1.2 levels negatively correlated with age in males. Conclusions: Adult GB displays a distinct circulating histone signature dominated by elevated free monomers rather than nucleosome complexes, contrasting with pediatric gliomas. These findings provide proof of concept for plasma histone monomers as novel minimally invasive diagnostic biomarkers in adult glioblastoma.

↑ Beginning

# Preservation, Disruption, and Acquisition of Cell-Type-Specific DNA Methylation in Cancer

To be considered for an Oral Presentation | 7. Epigenetic biomarkers

**Main author**: Dr. Yitzhak Reizel (Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Haifa, Israel · Assistant Professor)

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**Abstract**:
Background Cell type-specific DNA methylation patterns encode cellular identity and are generally maintained across mitotic divisions. These patterns are also known as powerful markers for cancer diagnosis. However, the methylome remains plastic and can be remodeled during development and disease through genetic, cellular, and environmental influences. A quantitative framework for assessing the stability of cell-identity-defining methylation signatures during tumorigenesis remains lacking. Methods Here, we applied a unified analytical framework across sorted bulk and single-cell methylome datasets. This approach consistently identified broad lineage signatures, including epithelial, leukocyte, and central nervous system lineages. It also identified more refined cell-type-specific signatures, such as hepatocyte-specific methylation patterns. We examined the dynamics of these signatures across 324 human cancers using different published genomic methylation platforms, including single-cell methylomes. Results In most cases, both broad and specific signatures were largely retained, indicating substantial preservation of the cell-of-origin signature despite malignant transformation. At the same time, we identified a subset of cancers with strong disruption of cell-of-origin methylation patterns. Strikingly, we found cell-type-specific signatures, such as those identified in a subset of neurons, that are acquired across multiple unrelated cancer types. Mechanistically, preserved tumor signatures are enriched for 5-hydroxymethylcytosine, indicating active preservation. Conclusions Collectively, our results reveal that tumors actively preserve cell-type-specific DNA methylation patterns, with disruption observed in only a minority of cases. Notably, the acquisition of unrelated epigenetic signatures highlights novel mechanisms of epigenetic reprogramming in cancer. Funding This study was supported by grants from the Israel-USA Binational Science Foundation (BSF), the Israel Cancer Research Fund (ICRF), the Israel Science Foundation (ISF), the Israel Cancer Association (ICA), the Israel Ministry of Innovation, Science and Technology (MOST), and the U.S. Department of Defense Congressionally Directed Medical Research Programs Prostate Cancer Research Program (CDMRP-PCRP).

↑ Beginning

# Towards Non-Invasive Detection of Ferroptotic Organ Injury via DNA Methylation Profiling

To be considered for an Oral Presentation | 7. Epigenetic biomarkers

**Main author**: Sr. Cyril Willemart (Cell Death Signaling lab, Infla-Med Centre of Excellence, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.)

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**Abstract**:
Ferroptosis is a regulated form of cell death driven by iron-dependent phospholipid peroxidation. As a major driver of acute organ injury across diverse etiologies, ferroptosis represents a high-value therapeutic target, as it can be pharmacologically inhibited to preserve tissue function. However, clinical translation is hindered by a lack of specific, high-resolution diagnostic tools. Emerging evidence indicates that cell death induction can trigger rapid DNA methylation changes, supporting the search for ferroptosis-specific methylation biomarkers. Despite its potential, a ferroptosis-specific methylome remains largely unexplored. We hypothesized that ferroptosis induces a specific DNA methylation signature that is distinguishable from other cell death modalities, providing a foundation for precision diagnostics. We performed native DNA methylation profiling using Oxford Nanopore sequencing across replicated in vitro and in vivo models. In vitro, ferroptosis was induced in murine hepatic cells (Hepa 1-6) using two distinct inducers. In vivo, ferroptosis was induced using a tamoxifen-inducible, liver-specific Gpx4 depletion mouse model. Additionally, apoptosis was induced via anti-Fas administration to enable assessment of ferroptosis specificity. Genome-wide analysis identified distinct differentially methylated regions (DMRs) associated with ferroptosis. In vitro profiling yielded ~30 high-confidence DMRs. Six genes associated with these DMRs were selected for further analysis, and their transcriptional levels were assessed in a kinetic qPCR experiment. This revealed a significant increase in transcript levels of X3 (a candidate histone demethylase) and X4 (an NAD-dependent protein deacetylase), consistent with promoter-associated hypomethylation. In vivo, ~20 high-confidence DMRs associated with ferroptotic liver injury were identified relative to both apoptotic and control conditions. Notably, a DMR linked to X3 was independently identified across both in vitro and in vivo models, supporting cross-model concordance of this candidate. We report previously uncharacterized DNA methylation changes associated with ferroptosis. Among these, X3 emerges as a lead candidate for further validation, supported by reproducible epigenetic changes, and concordant transcriptional activation. Ongoing work will extend validation to additional liver injury models to assess specificity and generalizability across etiologies. Furthermore, plasma cell-free DNA methylation profiling will be explored as a proof-of-concept for non-invasive detection of ferroptosis.

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www.zakladyboleslawiec.com

**Company description:**

Zakłady Ceramiczne “BOLESŁAWIEC” have been creating ceramics for nearly 80 years, combining the region’s centuries-old heritage with contemporary design.
As the oldest and largest ceramics manufacturer in Bolesławiec, the company proudly continues its tradition of craftsmanship. An important part of the company’s heritage is the traditional hand-stamping decoration technique, which has inspired hundreds of decorative patterns that contribute to the unique character of ZC “BOLESŁAWIEC” products. Today, the company is led by CEO Arkadiusz Grzesikowski and Board Member Wojciech Huczyński, who continue to preserve the brand’s distinctive identity while strengthening its position in Poland and worldwide.