Cole Foundation

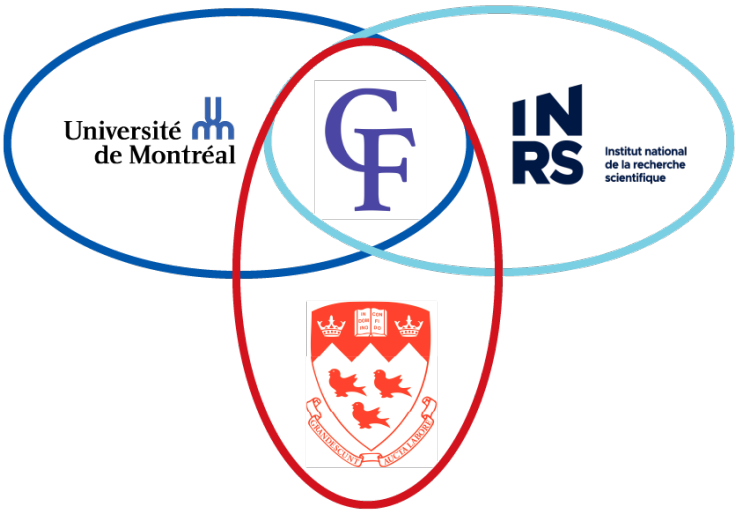
«Research Celebration Day»

20th Year

Journée «Célébrons la recherche»

de la Fondation Cole

20e Année



The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD programs to promote research in pre-leukemia, leukemia, and other leukemia-related diseases in children and young adults, as well as the development of clinical care for patients affected by these diseases. With the announcement of 2026 Fellows, the Fellowship program has supported more than 240 researchers in laboratories and hospitals situated in the Greater Montreal area through collaborations with l'Université de Montréal, McGill University, and INRS -Institut Armand-Frappier Research Centre.

10.6 million dollars have been invested in the fellowship program since inception. The Cole Foundation was established in 1980 by John N. (Jack) Cole as a private family foundation to provide funds for medical facilities concerned with childcare and research towards a cure for leukemia. The Foundation also awards grants to worthy community causes. The catalyst for the Foundation's creation was the death of his daughter, Penny Cole, from leukemia over a decade earlier. Through the Foundation, Jack Cole provided millions of dollars for medical facilities in Montreal. Notable among his achievements was the support of the Penny Cole Laboratory at the Montreal Children's Hospital and the establishment of the Jack Cole Chair in Pediatric Oncology and Hematology at McGill University.

Since his death in 2004, the Foundation has continued to carry on Jack Cole's mission with a renewed focus on research and childcare for young patients afflicted with leukemia and leukemia-related diseases.

The Cole Foundation

- **Ms. Nancy Wells** – President
- **Mr. Barry Cole** – Chair
- **Dr. Claude Perreault** – Board Member
- **Dr. Pierre Boyle** – Board Member
- **Dr. Pierre Chartrand** – Board Member
- **Ms. Gabrielle Cole** – Board Member
- **Ms. Viviane Cole** – Board Member
- **Mr. Charles K. Kaplan** – Board Member
- **Mr. David Laidley** – Board Member
- **Ms. Anne Lewis** – Board Member
- **Dr. Evan Lewis** – Board Member
- **Mr. Bruce McNiven** – Board Member
- **Dr. Guy Rouleau** – Board Member
- **Ms. Emma Tibaldo** – Board Member

La Fondation Cole

La Fondation Cole soutient la recherche sur la pré-leucémie, la leucémie et d'autres affections liées à la leucémie chez les enfants et les jeunes adultes, ainsi que le développement des soins cliniques pour les personnes atteintes de ces maladies, en offrant des bourses à des chercheurs-cliniciens, des résidents postdoctoraux et des étudiants diplômés inscrits à des programmes de doctorat. Avec l'annonce des boursiers 2026, le programme a appuyé plus de 240 chercheurs répartis dans des laboratoires et des hôpitaux de l'agglomération montréalaise, à la faveur de collaborations avec l'Université de Montréal, l'Université McGill et le Centre INRS - Institut Armand-Frappier. Depuis sa création, 10,6 millions de dollars ont été investis dans le programme de bourses.

La Fondation Cole a été instituée en 1980 par John N. (Jack) Cole à titre de fondation familiale privée pour subventionner des services de santés intéressant aux soins et à la recherche en pédiatrie et à la découverte d'un traitement curatif pour la leucémie. Elle a été créée à la mémoire de sa fille, Penny Cole, emportée par la leucémie plus de dix ans avant. La Fondation appuie aussi des causes communautaires louables. Grâce à la Fondation, Jack Cole a donné des millions de dollars à des services de santé de Montréal. Parmi ses grandes réalisations figurent son soutien du Laboratoire Penny Cole à l'Hôpital de Montréal pour enfants et la création de la chaire Jack Cole en oncologie-hématologie pédiatrique à l'Université McGill.

Depuis le décès de son maître d'oeuvre en 2004, la Fondation poursuit sa mission avec un intérêt marqué pour la recherche et les soins pédiatriques destinés à de jeunes patients affectés par la leucémie ou une maladie liée à la leucémie.

La Fondation Cole

- **Mme Nancy Wells** – Présidente
- **M. Barry Cole** – Chef du conseil
- **Dr Claude Perreault** – Membre du conseil d'administration
- **Dr Pierre Boyle** – Membre du conseil d'administration
- **Dr Pierre Chartrand** – Membre du conseil d'administration
- **Mme Gabrielle Cole** – Membre du conseil d'administration
- **Mme Viviane Cole** – Membre du conseil d'administration
- **M. Charles K. Kaplan** – Membre du conseil d'administration
- **M. David Laidley** – Membre du conseil d'administration
- **Mme Anne Lewis** – Membre du conseil d'administration
- **Dr Evan Lewis** – Membre du conseil d'administration
- **M. Bruce McNiven** – Membre du conseil d'administration
- **Dr Guy Rouleau** – Membre du conseil d'administration
- **Mme Emma Tibaldo** – Membre du conseil d'administration

THE COLE FOUNDATION

In association with
Université de Montréal & McGill University

presents its **"Research Celebration Day"**

in support of pediatric and young adult
leukemia and lymphoma research

Friday, May 15th, 2026

10:00 a.m. - 12:00 p.m.

Fellowship Poster Exhibition

12:00 p.m. - 1:00 p.m.

Lunch

1:00 p.m. - 2:00 p.m.

New Voices – New Ideas

- **Dr. Benjamin Martin** – Lady Davis Research Institute at the Jewish General Hospital

"Paralogue-specific functions of p300 acetylation in acute myeloid leukemia"

- **Dr. Marissa Rashkovan** – Centre de Recherche Azrieli du CHU Sainte-Justine

"Fueling Leukemia: Targeting Metabolic Vulnerabilities in Pediatric T-ALL"

- **Dr. Thomas Pincez** – Centre de Recherche Azrieli du CHU Sainte-Justine

"Single-cell level analysis of the effect of hydroxyurea on clonal hematopoiesis and hematopoietic stem cells in sickle cell disease patients"

2:00 p.m. - 3:15 p.m.

Keynote speaker: Dr. John E. Dick PhD FRS,

Professor, Molecular Genetics, University of Toronto; Senior Scientist, Princess Margaret Cancer Centre (UHN); Helga & Antonio De Gasperis Chair in Blood Cancer Stem Cell Research

"What makes a stem cell a stem cell and how does it go bad?"

Dr. John Dick is a world leader in the study of human hematopoietic stem cells. His development of the first human xenotransplant assay for normal and leukemic stem cells revolutionized stem cell research. His numerous contributions to human blood stem cell biology include the identification of repopulating stem cells, the discovery of different classes of stem cells, and the development of stem cell gene transfer. His landmark papers on the first identification of leukemic stem cells profoundly altered understanding of cancer biology. The recipient of numerous awards, we are delighted to have Dr. Dick with us to mark the 20th anniversary of the Cole Foundation's Research Celebration Day.

A reception will follow.

Address:

Pavillon Jean-Coutu

2940, Chemin de la polytechnique, Montréal

Eulalie Corre

Supervisor: Tarik Möröy

Title: Investigating the Impact of TET2 Mutations on AML Response to LSD1 Inhibition and Synthetic Lethal Vulnerabilities

Abstract: This project investigates epigenetic mechanisms altered in Acute Myeloid Leukemia (AML) cells carrying *TET2* mutations. Our study aims to identify new targets to improve epigenetic therapies by assessing the effects of *TET2* mutations on LSD1 inhibition and by finding new relevant therapeutic combinations.

Falak Khasawneh

Supervisor: François Mercier

Title: The role of ZFP36L2 in normal and malignant hematopoesis

Abstract: Understanding how Acute myeloid leukemia evades therapy requires targeting leukemic stem cells that drive resistance and relapse. This project aims to define the role and function of the RNA-binding protein ZFP36L2 in leukemic and normal hematopoietic stem cells, to identify therapeutically actionable vulnerabilities.

Yasamin Nassimi

Supervisor: Maureen McKeague

Title: Developing RNA-Based Differentiation Therapies for Acute Myeloid Leukemia

Abstract: Acute myeloid leukemia remains clinically challenging, particularly in pediatric settings, with limited durable targeted therapies. This work develops mRNA-based strategies to restore C/EBPα function, promoting leukemic differentiation and enabling safer, more precise treatment.

Johanna Toth

Supervisor: Anastasia Nijnik

Title: MYSM1 as a Therapeutic Target to Regulate Stress Response Signaling in MYC-Driven B-Cell Lymphoma.

Abstract: MYC is a key oncogene in lymphoma and we showed that MYSM1 cooperates with MYC in gene regulation in lymphoma cells. By defining how MYSM1 supports tumour growth and immune evasion, this work aims to develop targeted therapies that are more effective and less toxic than existing regimens.

Amirali Vahedi

Supervisor: François Mercier

Title: Molecular characterization, longitudinal immune profiling, and identification of vulnerabilities in residual acute myeloid leukemia after chemotherapy

Abstract: Acute myeloid leukemia is an aggressive blood cancer that often relapses after chemotherapy presumably due to measurable residual disease (MRD). This project will isolate and profile MRD and immune cells across treatment timepoints to uncover resistance mechanisms, immune vulnerabilities, and novel therapeutic targets to prevent relapse and improve patient outcomes.

UNIVERSITÉ DE MONTRÉAL

Yasmine Adda-Bouchard

Supervisor: Moutih Rafei

Title: Fumarate-based metabolic reprogramming of MSCs for Pediatric T-Cell Lymphoma immunotherapy

Abstract: This project investigates fumarate-driven metabolic reprogramming of mesenchymal stromal cells to enhance innate immune activation and antigen-presenting capacity. These cells are developed as a cellular vaccine platform to stimulate anti-tumor immune responses and enable innovative immunotherapeutic strategies for pediatric T-cell leukemia and lymphoma.

Milad Ahmadi Najfabadi

Supervisor: Christopher E. Rudd

Title: Enhancing Anti-CD19 CAR-T Efficacy via Rasal1 Downregulation

Abstract: Chimeric antigen receptors have proven effective for cancer immunotherapy. We identified Rasal1 as a novel negative regulator of T-cell activation associated with antigen receptor signaling. This project enhances anti-CD19 CAR-T therapy through rational CAR design with unique hinge regions and Rasal1 downregulation to potentiate targeting of acute lymphoblastic leukemia (ALL).

Arnau Ballestero Vidal

Supervisor: Hélène Decaluwe
Title: Modulating IL-2Rβ Signaling to Preserve Stem-Like $CD8^+$ T Cells for Enhanced CAR-T Therapy

Abstract: T-cell exhaustion is a major limitation in CAR-T therapy, often causing relapses. By targeting the IL2RB-STAT5 pathway during manufacturing, this project aims to preserve stem-like progenitor T-cells, associated with enhanced persistence and function. Using spectral flow cytometry and murine models, we will demonstrate how this strategy improves long-term tumor control.

Ophélie Dézé

Supervisor: Sonia Cellot

Title: Identify key mediators of sensitivity and resistance to BCL-XL inhibitors in AML

Abstract: Here is my summary my research project : My research aims to identify the mode of action of the pro-survival factor BCL-XL inhibitors, in acute megakaryoblastic leukemia by using the CRISPR-Cas9 screen technology. This research will pave the way for potential future therapeutic approaches, adapted to the heterogeneity of AMKL patients and effective in understanding the mechanism of apoptotic pathways in pediatric AMKL.

Elham Ebrahimi

Supervisor: Christopher Rudd
Title: ADAP-Engineered CAR-T Cells for Enhanced Therapy in Pre–B Acute Lymphoblastic Leukemia

Abstract: My research focuses on enhancing CAR-T cell therapy by integrating ADAP-mediated signaling to improve T-cell activation, adhesion, and persistence, with the goal of generating more effective and durable treatments for pediatric and young adult leukemia.

Esteban Gomez Vivolo

Supervisor: Joey Ghersi
Title: Elucidating the developmental origin of pediatric leukemia induced by sh2b3 loss.

Abstract: My research aims to investigate how the loss of SH2B3, a key regulator of JAK/STAT signaling in hematopoietic stem and progenitor cells and a gene frequently mutated in pediatric leukemia, contributes to the establishment of a pre-leukemic state during embryonic development. This process likely occurs well before the clinical onset of pediatric leukemia. I aim to identify early biomarkers of

pediatric leukemia, thereby refining preventive strategies and improving therapeutic approaches for this type of cancer.

Malak Lahrichi

Supervisor: Moutih Rafei

Title: Succinate-based reprogramming of mesenchymal stromal cells into potent cancer vaccine for lymphoma

Abstract: Cell-based cancer vaccines aim to induce an anti-tumoral response by infusing Antigen Presenting Cells (APCs) into patients. Our laboratory explores the use of Mesenchymal Stromal Cells (MSCs) as an alternative to Dendritic Cells (DCs). My project focuses on the metabolism of MSCs and how it can be leveraged to enhance MSC-mediated antitumor immune responses, supporting the development of MSC-based cancer vaccines as a novel off-the-shelf immunotherapeutic strategy.

Perla Matar

Supervisor: Moutih Rafei

Title: ESCiCeLL: An Endosomal-Escape MSC Vaccine Platform for Therapeutic Lymphoma Immunotherapy

Abstract: Developing a novel mesenchymal stromal cell (MSC)-based cancer vaccine (ESCiCeLL) to enhance the immune system's ability to eliminate residual cancer cells. This work aims to improve T-cell responses and establish a scalable immunotherapy approach to reduce relapse in leukemia and lymphoma.

Mojtaba Mollaei

Supervisor: Hua Gu

Title: The role of Transcription Factor EB (TFEB) in regulating T cell subsets against T-cell Lymphoma (TCL)

Abstract: T-cell lymphoma (TCL) is a subset of aggressive lymphomas which still does not have an effective cure. Recently, we have found that transcription factor EB (TFEB) controls an immune checkpoint which prevents CD8+ T cells from response to and kill of the EG7 tumor (a T cell lymphoma model). This finding thus warrants further studies to explore whether targeting TFEB in T cells is an effective approach to treat TCL.

Marcelo Muniz Corrêa

Supervisor: John M.Pascal

Title: Assessment of next-generation PARP inhibitors in poly(ADP-ribose)-mediated cell death in acute myeloid leukemia therapeutics

Abstract: The poly(ADP-ribose) (PAR)-dependent cell death pathway parthanatos has been associated with better survival in acute myeloid leukemia (AML) patients treated with frontline chemotherapy. In this project, we will evaluate the contribution of protein-free PAR to parthanatos in AML cell lines, aiming to profile leukemia subtypes for precise combination therapy.

Éléonore Provencher

Supervisor: Marissa Rashkovan

Title: Targeting Phosphatidylcholine Metabolism in T-ALL

Abstract: T-cell acute lymphoblastic leukemia (T-ALL) is a high-risk leukemia, and despite therapeutic advances, survival rate of relapse patients remains low, at only 20%. My project aims to characterize phospholipid metabolism in T-ALL and evaluate the therapeutic potential of CHKA inhibition as a potential new treatment strategy.

Émilie Trudel

Supervisor: Léandra Desjardins

Title: Comment ça va, papa? Un portrait de la santé mentale des pères en oncologie pédiatrique

Abstract: Fathers with a child with cancer are underrepresented in mental health research. This project aims to better understand the impact of pediatric cancer on fathers' mental health and their use of psychosocial services. Results from this research will guide the development of services that meet their needs.

Title: Dual-Modality Engineering of CAR T Cells via Hinge Optimization and Mediator Inhibition Enhances Antitumor Efficacy

Authors: Milad Ahmadi Najafabadi[1-3], Fatemeh Yousefi[1-3,4], Xueyang Guo[1-3] and Christopher E. Rudd[1-5]

Affiliation: [1]Département of Medicine, Universite de Montréal; [2]Centre de Recherche Hôpital Maisonneuve-Rosemont; [3]Department of Microbiology, Infection and Immunology, Universite de Montreal; [4]ImmunAb Research Inc.; [5]Institut Universitaire d'Hématologie-Oncologie et Thérapie Cellulaire de Montréal, Hôpital Maisonneuve-Rosemont,

Keywords: CAR T-cell therapy; DLBCL; hinge optimization; shRNA; RAS signaling.

Background Information: Chimeric antigen receptor (CAR) T-cell therapy has achieved significant clinical success in hematologic malignancies; however, its long-term efficacy remains limited by insufficient persistence and progressive functional exhaustion, particularly within the tumor microenvironment. These challenges are especially relevant in pediatric cancers, where durable and safe therapeutic strategies are critically needed. Diffuse large B-cell lymphoma (DLBCL), including the A20 model, represents an aggressive subtype accounting for approximately 10–20% of childhood non-Hodgkin lymphomas and remains a key target for improved immunotherapies. Building on our laboratory's work defining proximal T-cell receptor (TCR) signaling, we have defined several keys signaling proteins that regulate T-cell function.

Purpose of the Study: Here, we investigated whether combining CAR hinge optimization with shRNA-mediated suppression of several of mediators could enhance CAR T-cell function and antitumor activity, with a focus on B-cell malignancies.

Methods: CD19-directed CAR T cells were engineered with hinge modifications and bicistronic shRNA constructs targeting selected signaling mediators. Functional efficacy was evaluated through in vitro assays and in vivo tumor models, including the A20 DLBCL model. T-cell activation, proliferation, cytokine production, and exhaustion marker expression were assessed, along with PCR-based profiling of inflammatory cytokines.

Results: Using CD19-directed CAR T cells, bicistronic knockdown significantly improved tumor control both in vitro and in vivo, with pronounced efficacy in the A20 DLBCL model. Enhanced antitumor activity was associated with increased T-cell activation and proliferation

(CD69, TCF-1, Ki-67), elevated effector cytokine production (TNF-α, IFN-ɣ), and reduced expression of exhaustion markers (PD-1, TIM-3) within tumor-infiltrating lymphocytes. Notably, PCR profiling revealed a reduction in broader inflammatory cytokine expression, suggesting a more controlled and potentially safer activation profile. Importantly, hinge-optimized CAR constructs combined with mediator suppression consistently outperformed conventional CAR designs across multiple tumor settings, demonstrating improved intratumoral persistence and sustained functional competence.

Conclusion: These findings highlight a novel combinatorial strategy to enhance CAR T-cell durability and efficacy. Collectively, this work underscores the therapeutic potential of integrating intrinsic signaling modulation with CAR structural optimization, particularly for aggressive pediatric B-cell malignancies such as DLBCL and supports the development of next-generation CAR T-cell therapies for childhood leukemias and lymphomas.

Title: The immunopeptidome in T-ALL is dynamic & shaped by drug treatment

Authors: Anleu Alegria, Javier[1], M. Tremblay[1], J. Lanoix[2], E. Bonneil[2], C. Durette[2], M. Courcelles[2], C.M. Pascariu[2], M.P. Hardy[3], Claude Perreault[3], Pierre Thibault[2], Trang Hoang[1].

Affiliations: Unités de recherche en hématopoïèse et leucémie[1], protéomique et spectrométrie de masse[2], et immunobiologie[3], Institut de Recherche en Immunologie et en Cancérologie, Université de Montréal.

Keywords: T-cell acute lymphoblastic leukemia, immunopeptidomics, drug treatment, cellular stress response.

Background Information: T-cell acute lymphoblastic leukemia (T-ALL) is among the most common pediatric cancers. Despite high cure rates, mainstay chemotherapy, comprising Vincristine, Dexamethasone, and L-Asparaginase (VXL), has reached the limit of dose intensification due to side effects that severely compromise patients' quality of life. Improving therapeutic strategies requires a better understanding of T-ALL biology. Tumour immune surveillance is shaped by MHC-I-associated peptides (MAPs) presented at the surface of tumour cells.

Purpose: Using transcriptomic and mass spectrometry analyses, we assessed whether and how VXL chemotherapy alters the MAP repertoire (immunopeptidome) of T-ALL cells.

Methodology & Results: Over-representation analyses of genes encoding MAPs (i.e., source genes) from seven T-ALL patient-derived xenografts revealed enrichment of cancer stress response pathways, which correlated with sensitivity to chemotherapeutic agents. Next, we

compared the MAP landscape of Jurkat T-ALL cells treated with VXL or DMSO (control) using immunopeptidomics mass spectrometry and RNA-seq. Our analyses showed that the chemotherapeutic drugs significantly remodel the immunopeptidome. Notably, the source genes of MAPs whose abundance changed significantly were enriched in cell cycle pathways, consistent with VXL's known mechanism of action. Together, these findings suggest that the immunopeptidome is dynamically modulated by chemotherapeutic stress and reflects the underlying cellular state.

Conclusion: Our integrative approach uncovers previously underappreciated immunopeptidomic changes induced by chemotherapy and highlights the untapped potential of immunopeptidomics to complement the toolbox for studying cell state. We postulate that integrating immunopeptidomics could open new perspectives for the development of both pharmacological and immunotherapy-based strategies.

Title: Impact of sucralose on cell metabolism and chemotherapy sensitivity in young leukemia patients

Authors: Dr. Léa Bourguignon[1], Elpida Bokou Gianneli[1], Lina Ameur[1]; Giulio Aceto[2], Dr. Sidong Huang[2], Dr. Julianna Blagih[1]

Affiliation: [1]Centre de Recherche de l'Hôpital Maisonneuve Rosemont, Faculty of Medicine, University of Montreal. [2] Department of Biochemistry, McGill University.

Keywords: T-ALL, human cell lines, artificial sweeteners, metabolism

Background information: T-cell acute lymphoblastic leukemia (T-ALL) is a highly aggressive and proliferative cancer accounting for 15% of pediatric and 25% of young adult ALL cases. While treatments have improved survival rates in children, many young adults still face challenges, and about 20% of all cases relapse or are refractory to treatment. To sustain unchecked proliferation, T-ALL cells upregulate nutrient uptake and cellular metabolism to meet their biosynthetic and energetic needs. In fact, current standard-of-care therapies, such as methotrexate (MTX), an antimetabolite that targets T-ALL metabolic pathways. Diet can also be harnessed to target the cellular metabolism of T-ALL cells. Sucralose, a commonly consumed artificial sweetener, has recently been found to impair proliferation in the T-ALL cell line Jurkat as well as key upstream signalling, how it affects cellular metabolism and whether this affects a wide range of T-ALL cells remains unknown.

Purpose of the study: The goal of the project is to understand how to exploit dietary sucralose to improve current treatments for T-ALL

Methods: Using six human T-ALL cell lines, we assessed the effects of sucralose and the structurally distinct artificial sweetener acesulfame

potassium (AceK). Cell proliferation was quantified by cell counting, and apoptosis was measured by flow cytometry using Annexin V/PI staining. Cell cycle progression was evaluated by EdU incorporation. Mitochondrial parameters, including mass and membrane potential, were assessed using MitoTracker Green and Red, respectively. Mitochondrial function was further evaluated using a Seahorse Mito Stress Test. To investigate the mechanisms underlying sucralose responses, we performed a genome-wide CRISPR knockout screen in Jurkat cells with or without sucralose exposure.

Results: We found that five of six T-ALL cell lines reduced cell growth in the presence of sucralose compared to AceK and vehicle, yet there were no obvious changes in cell cycle progression. Of the sucralose-sensitising lines, we observed that sucralose induced cell death, accompanied by increased mitochondrial mass and no change in membrane potential, suggesting reduced mitochondrial activity. Indeed, a MitoStress Test revealed that sucralose modulates mitochondrial activity in T-ALL cells. Sucralose further sensitised T-ALL cells to apoptosis in the presence of mitochondrial stressors: the mitochondrial uncoupler FCCP and the antimetabolite MTX. Furthermore, the CRISPR-KO screen supported the conclusion that sucralose induces mitochondrial vulnerabilities, which could be harnessed by targeting these susceptibilities.

Conclusion: This project shows that sucralose impairs proliferation in T-ALL cell lines by increasing apoptosis without disrupting the cell cycle. Interestingly, sucralose was found to affect mitochondrial homeostasis and increase T-ALL sensitivity to mitochondrial stressors, including FCCP and MTX. This project is a first step toward improving the treatment of T-ALL.

Title: Enhancing isoform detection in AML using *ad hoc* references and long-read scRNA-seq

Author: Anshul Budhraja

Affiliation: Dr. Vincent-Philippe Lavallée, Centre de recherche du CHU Sainte-Justine / Université de Montréal

Keywords: Single cell RNA-seq, Acute Myeloid Leukemia, Long-read RNA-seq, Transcriptomics and Bioinformatics

Background information: Acute Myeloid Leukemia (AML) is a hematological malignancy characterized by the accumulation of somatic mutations in hematopoietic stem cells, leading to the disruption of normal blood cell development. Despite significant advances in genetic and transcriptomic profiling, AML remains frequently fatal due to the complexity of its molecular landscape. Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool to resolve cellular diversity

and identify aberrant phenotypes, providing insight into disease heterogeneity, treatment resistance, and rare leukemic populations. However, conventional short-read scRNA-seq relies on fragmented reads and static transcriptomic references, limiting its ability to resolve full-length transcripts, fusion events, and isoform diversity. As different isoforms of the same gene can produce distinct proteins and alter cellular behavior, accurately characterizing transcript structure is essential for understanding AML biology.

Purpose: This study aims to improve the detection and characterization of transcripts and isoforms in AML using long-read single-cell RNA sequencing, with a focus on incorporating sample-specific (*ad hoc*) transcript references.

Methods: Five AML samples from the Leucegene cohort were sequenced using both long-read (Oxford Nanopore) and short-read (Illumina) scRNA-seq from matched libraries. Short-read data were processed using Cell Ranger, while long-read data were analyzed using wf-single-cell. To address limitations of static references, transcript assemblies were produced using StringTie3 and IsoQuant. These assemblies were used as *ad hoc* references, i.e. references derived directly from the sequenced data. Comparative analyses were performed to evaluate transcript detection, isoform resolution, and quantification. In addition, assembled transcripts from AML samples were examined to identify novel transcript structures, and to assess their representation in known annotations.

Results: Long-read scRNA-seq captures the major biological signals observed in short-read data while providing additional resolution at the level of transcript isoforms. However, current long-read analysis pipelines introduce biases through stringent filtering and reliance on incomplete reference annotations, resulting in the loss of biologically relevant transcripts. Using *ad hoc* references improves transcript detection and reveals isoforms that are not represented in standard annotations. Analysis of AML assemblies demonstrates the presence of previously unannotated transcript structures, including isoforms with distinct expression patterns across cellular populations. Notably, we observe different isoforms of the same gene display opposing expression patterns within hematopoietic stem-like cells, highlighting the importance of isoform-level resolution in understanding leukemic biology.

Conclusion: *Ad hoc* reference construction enhances the analytical power of long-read single-cell RNA sequencing by enabling the recovery of sample-specific and isoform-resolved transcriptomes in AML. By directly examining assembled transcripts, this approach reveals layers of transcriptomic complexity that are missed by conventional methods. These findings support a shift toward dynamic, sample-specific

transcriptome annotation and provide a framework for improved molecular characterization of AML. Ultimately, better detection of isoform-level variation may contribute to more accurate disease classification and the identification of novel therapeutic targets.

Title: An Ongoing Systematic Review of SelfManagement Support and Remote Symptom Monitoring Programs after an Allogeneic Hematopoietic Stem Cell Transplantation

Authors: *Sarah Chehayeb[1] RN, PhD candidate, Dr. Sylvie Lambert[1,2] RN, PhD.

Affiliations:

1: Ingram School of Nursing, McGill University.

2: St. Mary's Research Centre, St. Mary's Hospital.

Keywords: Allogeneic hematopoietic stem cell transplantation (allo-HSCT), hematologic cancers, self-management (SM), self-management support (SMS) programs, remote symptom monitoring (RSM) programs.

Background information: Allogeneic hematopoietic stem cell transplantation (alloHSCT) can cure hematologic cancers but remains high-risk due to graftversushost disease, infections, and mortality. After discharge, patients and caregivers must manage significant symptoms at home with limited guidance, and weekly or biweekly followups leave gaps where complications may go unnoticed. Selfmanagement support (SMS) programs help address symptoms and prevent complications. Remote symptom monitoring (RSM) enables electronic symptom reporting between visits through patientreported outcome measures (PROMs), such as 0–10 ratings, to prompt timely clinician feedback. SMS and RSM have improved outcomes in the broader cancer population. Yet, no review has synthesized SMS and RSM programs in the alloHSCT context, limiting the ability to identify and address existing care and research gaps.

Purpose of the study: 1) Synthesize existing post–alloHSCT SMS and RSM programs according to the Template for Intervention Description and Replication (TIDieR) checklist.

Methods: Ongoing scoping review. PsycINFO, MEDLINE, Embase Classic + Embase, Scopus, CINAHL, and the Cochrane Library were searched using keywords related to allo-HSCT, self-management (SM), SMS and RSM. Inclusion criteria were SMS and /or RSM programs developed for patients and/or caregivers post-allo-HSCT. Data were extracted and analyzed using the TIDieR checklist: why, what, who, how, where, when, tailoring, and how well.

Results: 5 patients, 2 caregivers, 1 dyadic program found. Why: patient and dyadic ones aimed to support SM. Caregiver ones focused on psychosocial strain/coping. What: patient programs were symptom-focused or multi-component; caregiver ones were psychosocial; dyadic targeted problem-solving. RSM was rarely integrated (n=1). None addressed all SM skills and tasks while also including caregivers. Who: delivered by nurses (n=4), advanced practice nurses/care coordinators (n=2), therapists (n=2), social workers (n=2), sports therapists (n=1), and multidisciplinary teams (n=2). How: Face-to-face (n=8), digital (n=4), phone/video (n=4), most hybrid (n=6). Where: inpatient (n=8), outpatient (n=6), home (n=4), most spanning multiple settings (n=5). When: pre-transplant (n=4), admission (n=8), post-discharge (n=6), most spanning multiple phases (n =7). Tailoring: most were tailored (n=5). How well: feasible/acceptable (n=6); adherence challenges (n=3); some outcome improvements (n=5).

Conclusion: Overall, existing post–alloHSCT SMS and RSM programs show several limitations. Many do not cover the full range of SM skills and tasks or address agespecific needs. Caregivers are rarely included, and caregiver programs overlook broader needs and place a substantial burden on participants. The only dyadic program reported high attrition, pointing to the value of lowerintensity online formats. Few programs draw on theoretical frameworks or incorporate behaviour change techniques. RSM is also rarely integrated, despite strong evidence of its benefits. These gaps need to be addressed in future program development for the allo-HSCT population, and program efficacy needs to be tested.

Title: Targeting mitochondrial dynamics in T-cell Acute Lymphoblastic Leukemia

Author: Juliette Durocher

Affiliation: Dr. Marissa Rashkovan, Département de pathologie et biologie cellulaire, CRA-CHU Sainte-Justine, Université de Montréal

Keywords: Acute lymphoblastic leukemia, Targeted therapy, Mitochondria, Metabolism

Background information: Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, accounting for about 25% of newly diagnosed childhood cancers. Amongst those cases, 15% are of the T-cell subtype (T-ALL), characterized by abnormal proliferation of immature T-cell precursors. Despite improvements in treatment protocols, 15% of T-ALL patients still relapse and the prognosis of these patients remains poor. This highlights the need for new therapeutic strategies.

Recently, mitochondria have been identified as an important factor mediating drug resistance in T-ALL, a major contributor of disease relapse. Preliminary data from the lab identified mitochondrial fusion protein MFN2 as essential for T-ALL survival, making it a good candidate target.

Purpose of the study: This project investigates the effects modifying mitochondrial dynamics homeostasis in T-ALL cells in efforts to find new targeted therapeutic strategies.

Methods: We used an inducible CRISPR-Cas9 system to generate a KO T-ALL cell line for mitochondrial fusion protein MFN2 to look at its impact on cell fitness. We also used pharmacological inhibition of mitochondrial fusion protein MFN2 to assess proliferation and survival in several T-ALL cell lines and Patient-derived xenografts. We then characterized the phenotype of these cells following MFN2 inhibition using a combination of RNA-sequencing, Western blots, flow cytometry and metabolic assays and confocal microscopy. Finally, we looked at the potential of MFN2 inhibition to synergize with current T-ALL therapies.

Results: We show that knocking out MFN2 impairs T-ALL proliferation in vitro and delays leukemia progression in vivo. We also show that pharmacological inhibition of this mitochondrial fusion gene successfully reduces proliferation and induces apoptosis of T-ALL cells. We also show reduced mitochondrial function, increased reactive oxygen species production and changes in the mitochondrial network of T-ALL cells following MFN2 inhibition. Lastly, we show that MFN2 inhibition synergizes with Venetoclax, a newly approved BCL2 inhibitor that has shown very promising results.

Conclusion: The results obtained here support the idea that mitochondria are central to T-ALL metabolism. They highlight targeting mitochondrial dynamics protein MFN2 as an option in T-ALL and will provide new future avenues for T-ALL therapies.

Title: Pharmacological reprogramming of mesenchymal stromal cells as a novel treatment modality for cancer

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Keywords: Mesenchymal stromal cells, Pharmacological modulation, Antigen presentation, Cancer antigens, Leukemia/Lymphoma

Background Information: Unlike normal cells, cancer cells undergo DNA modifications leading to the expression of tumor-associated antigens (TAAs). In fact, multiple screening studies corroborate their elevated

expression levels in leukemia/lymphoma patients. Thus, *de novo* induction of these antigens in cellular vaccines constitutes an interesting target for cancer immunotherapy. Our team has already demonstrated how mesenchymal stromal cells (MSCs) could be genetically and pharmacologically engineered into antigen presenting cells (APCs); accordingly, we aim to reprogram MSCs to present cancer-associated antigens in order to mount a cancer-specific immune response.

Purpose of the study: To assess the capacity of pharmacologically reprogrammed MSCs to express and present various cancer antigens (e.g. behave as antigen-presenting cells) as well as their potential use as a novel cancer vaccine platform.

Methods: A series of incubation studies using different doses for various durations were performed to determine the most tolerated doses (MTD) of multiple pharmacological agents and the optimal treatment duration yielding the most efficient increase in cell surface MHC class I expression with the least apoptosis. An RNA-seq along with an immunopeptidome analysis were conducted to investigate the overall transcriptome and the drug-induced effect on antigen-derived peptide repertoire respectively. Finally, the therapeutic and prophylactic efficacy of reprogrammed MSCs were assessed against various cancer cell types (EL4, E0771, X577, Pan02, and B16) in immunocompetent C57BL/6 mice.

Results: The safety and tolerability of 7 *ex vivo* pharmacological treatments have already been confirmed. The MTD of each drug was determined following 48h and 72h treatments and the concentrations inducing tolerable apoptosis were identified. MHC class I quantification following treatments revealed increased levels in a dose-dependent manner. More importantly, 2 out of the 7 tested drugs induced *de novo* expression of ~40 TAAs each in treated MSCs which abrogated B16 and EL4 tumor growth in 60% and 40% of prophylactically vaccinated mice respectively. The therapeutic potency of this vaccine is still being tested.

Conclusion: This research could enhance our understanding of certain cancer antigens and has demonstrated their efficacy as targets for cancer immunotherapies. Moreover, our work could result in the development of innovative, broad-ranged and simple to manufacture off-the-shelf vaccines, which could be used as both prophylactic and therapeutic immunotherapies.

Title: *R*-2-hydroxyglutarate-mediated inhibition of KDM4A disrupts telomere replication integrity

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Keywords: *R*-2-hydroxyglutarate, IDH mutations, telomere, KDM4A, DNA replication

Background information: Pediatric acute myeloid leukemia (AML) is the second most common leukemia detected in children and can arise from various genetic mutations or chromosomal abnormalities, including mutations in isocitrate dehydrogenases (IDH1/IDH2). Recent studies suggest that IDH mutations in pediatric AML may correlate with a poorer prognosis. These mutations lead to the production of the oncometabolite *R*-2-hydroxyglutarate (*R*-2HG) instead of the usual product, αketoglutarate (αKG). *R*-2HG, structurally similar to αKG, acts as an inhibitor of αKG-dependent enzymes, such as the JUMONJI family of lysine demethylases, with KDM4A being particularly affected. However, the precise mechanism by which *R*-2HG-mediated inhibition promotes AML is not fully understood. Our preliminary results show that KDM4A localizes to telomeres, and that its depletion or inhibition by *R*-2HG leads to telomere defects, suggesting a role for KDM4A in telomere stability.

Purpose of the study: We hypothesize that *R*-2HG induces telomere dysfunction by impairing DNA replication at telomeres through inhibition of the KDM4A catalytic domain, thereby promoting genomic instability in blast cells that contribute to AML development.

Methods: To evaluate the impact of IDH1/2 mutation, which produce the oncometabolite *R*-2HG, on telomeres, we used cells transduced with $IDH1^{WT}$ or $IDH1^{Mutated}$ or treated with *R*-2HG. Telomere integrity was assessed by FISH on metaphase spreads. DNA replication was evaluated using DNA fiber assays and EdU incorporation, followed by FISH/IF staining. Similar experiments were conducted in cells depleted of KDM4A or treated with KDM4 inhibitors (QC6352 and ML324). To investigate KDM4A association with telomeres, we performed ChIP-seq, immunoprecipitation, and GST pull-down assays.

Results: We demonstrate that exposure to *R*-2HG or KDM4A depletion induces senescence, in a p53-dependent manner. In addition to localizing at telomeres, KDM4A regulates levels of telomeric H3K9me3. KDM4A also interacts with the shelterin complex, particularly with TRF2 through its PHD domain. Rescue experiments using either a catalytically inactive mutant or a PHD domain-deficient KDM4A fail to restore

telomeric defects compared to KDM4A WT, suggesting that both the binding to the shelterin complex and the catalytic activity of KDM4A are essential for telomere stability. Furthermore, cells exposed to *R*-2HG or depleted of KDM4A exhibit reduced replication forks progression. Depletion of SMARCAL1, a helicase involved in replication fork reversal, mitigates telomeric defects induced by *R*-2HG or KDM4A inhibition with QC6352. This result correlates with decreased SMARCAL1 expression in IDH1/2-mutated tumors compared to IDH1/2 wild-type cancers.

Conclusion: This study elucidates the role of methylation in telomeres regulation and improve our knowledge of the early events leading to AML related to the effect of genomic instability on telomeres.

Title: Improving the Well-Being of Adolescent and Young Adult Survivors of Pediatric Hematological Cancers: Mapping Behaviour Change Interventions and Identifying Determinants of Substance Use

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Keywords: Adolescent and Young Adult, Pediatric Hematological Cancers, Health Behaviours, Alcohol Use, Tobacco Use

Background: Adolescent and young adult survivors of childhood hematological cancers (AYA-HCCS) face significantly elevated risks of chronic health conditions and treatment-related late effects. While adopting healthy lifestyle behaviours is critical to reducing morbidity and mortality, this population remains vulnerable, particularly concerning substance use. A gap remains in understanding the effectiveness of current interventions and the specific determinants of high-risk behaviours, such as tobacco and alcohol consumption within this demographic.

Purpose: This research follows a two-fold objective: 1) to map and categorize existing health behaviour change interventions for AYA-HCCS through a scoping review (Study 1); and 2) to identify the behavioural and informational factors associated with alcohol and tobacco use to inform future nurse-led health promotion strategies (Study 2).

Methods: The first phase involved a systematic scoping review following the Joanna Briggs Institute (JBI) guidelines, analyzing 22 studies using Michie's (2018) Behaviour Change Wheel framework. The second phase

(cross-sectional study) utilizes an online survey based on the COM-B and Transtheoretical models. It targets 200 survivors (aged 18–39) recruited via the CHU Sainte-Justine long-term databank and community partners to assess alcohol and tobacco consumption patterns, readiness to change, and perceived barriers/facilitators.

Results: The scoping review revealed a heavy focus on physical activity (n=15/22) and a strong reliance on educational and training functions, largely overlooking tobacco and alcohol use. Expected results from the cross-sectional study will identify key behavioural profiles, perceived health vulnerability, and highlight gaps in the guidance provided by healthcare professionals.

Conclusion: Findings emphasize the need to diversify intervention strategies beyond simple education by integrating techniques such as environmental restructuring or incentivization. By identifying the specific needs of AYA-HCCS, this project provides a foundation for developing comprehensive, multidimensional, and nurse-led health promotion programs aimed at improving long-term health outcomes and quality of life. The integrated results will support the development of tailored, promotion strategies in hematology-oncology survivorship clinics.

Targeting eIF4A to disrupt adaptive survival programs and metabolic plasticity in venetoclax/5-azacitidine resistant acute myeloid leukemia

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Keywords: Acute myeloid leukemia (AML); drug resistance; targeted therapy and chemotherapy; eIF4A; metabolism

Background: A major obstacle in AML clinical management is the development of resistance to targeted therapies, driven by adaptations that bolster metabolic plasticity and anti-apoptotic protein expression. In the relapsed setting, mTORC1 and RAS signaling pathways are often hyperactivated due to somatic mutations and drive cap-dependent translation of oncogenic mRNAs by regulating assembly of the eukaryotic initiation factor 4F (eIF4F). Our previous work identified pharmacological inhibition of the mRNA helicase subunit of the complex, eIF4A, as a

key vulnerability. The therapeutic potential of eIF4A targeting is underscored by the development of inhibitors, such as Zotatifin (eFT226), currently tested clinically. We hypothesize that pharmacological inhibition of eIF4A prevents therapeutic adaptations across diverse AML genotypes by selectively suppressing the translation of key metabolic and anti-apoptotic drivers.

Methods: We utilized a panel of AML cell lines with diverse oncogenic drivers. We measured apoptosis rates, anti-apoptotic protein expression, and mitochondrial states using flow cytometry. *In vivo*, a PDX model (*DNMT3A*, *NPM1.FLT3*^{ITD}, *IDH2*) was treated for 28 days with a triple combination of zotatifin, venetoclax, and 5-azacytidine (Ven/Aza + zota), followed by a 4-month observation period. To dissect resistance mechanisms, primary AML cells harvested post-Ven/Aza treatment were analyzed *ex vivo* to quantify anti-apoptotic proteins (MCL-1, BCL-2, BCL-XL) and associated survival signaling (p-STAT5, p-4EBP1, p-RPS6, p-ERK-1/2) via flow cytometry.

Results: Among the cells tested, eIF4A inhibition significantly reduces anti-apoptotic protein levels (BCL-2), triggering programmed cell death and disrupting bioenergetic stability. We highlight a potential compensatory mechanism characterized by an upregulation of MCL-1 expression. *In vivo*, the Ven/Aza + zota combination achieved complete and long-lasting AML eradication with no relapse observed during the 4-month follow-up. Conversely, cells persisting after standard Ven/Aza therapy displayed a significant increase in BCL-2 expression and associated survival signaling (p-STAT5, p-RPS6) suggesting resistance through either clonal selection of BCL-2^{high} cells or transient compensatory upregulation of BCL-2.

Conclusion: Our findings demonstrate that targeting eIF4A-mediated translation initiation can impair anti-apoptotic proteins expression in AML cells to prevent escape from Ven/Aza therapy. Ongoing work will expand these findings to a diverse cohort of primary AML specimens to evaluate therapeutic potential across a larger set of oncogenic drivers. To identify biomarkers of response, we will implement pharmacogenomic CRISPR and ORF screens. Additionally, we are performing in vivo LC/MS analyses to define the specific metabolic shifts driven by eIF4A that facilitate leukemic survival under therapeutic pressure.

Title: Histone H3-K27M mutation drives pre-leukemia by reactivating a fetal hematopoietic gene signature

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Keywords: AML, pre-leukemia, H3-K27M mutation, onchohistones

Background: Acute myeloid leukemia (AML) is a highly aggressive and genetically complex hematological malignancy. Despite advances in its understanding, many aspects of AML progression remain elusive. We previously demonstrated that the histone H3 lysine-27-to-methionine (H3-K27M) onchohistone drives a pre-leukemic state in human hematopoietic stem and progenitor cells (HSPCs), characterized by enhanced self-renewal, myeloid lineage skewing, and an erythroid differentiation block. Our unpublished single-cell RNA sequencing data of xenograft models show that H3-K27M reactivates a fetal hematopoietic gene signature in adult HSCs suggesting that this mutation induces a developmental reprogramming toward a fetal-like state associated with increased self-renewal capacity that predisposes to transformation.

Purpose: In this study, we further investigate the molecular mechanisms underlying H3-K27M-driven pre-leukemia through CRISPR/Cas9-mediated knockout of upregulated fetal-associated genes.

Methods: To accelerate this project, we optimized an *in vitro* culture system that recapitulates the H3K27M-associated phenotypes previously observed in our xenograft model. For fetal gene knockout experiments, we established a CRISPR/Cas9-based approach. After designing and validating guide RNAs in cord blood cells, we selected the most efficient guides to target candidate fetal genes. These candidate genes were then knocked out in human cord blood HSPCs in the context of either H3-K27M or wild-type (WT) backgrounds and cultured *in vitro* for two weeks. Cellular phenotypes were assessed by flow cytometry, including stem and progenitor cell frequencies, lineage distribution, and differentiation status, to determine whether gene knockout could rescue the H3-K27M-associated phenotype. In addition, colony-forming unit (CFU) assays were performed to evaluate the impact of fetal gene knockout on progenitor cell's function.

Results: We demonstrate in vitro that H3-K27M mutation significantly increases the number of HSCs and alters erythroid dynamics with a promotion of early erythroid commitment while impairing terminal maturation. Deletion of *PLAG1* and *LIN28B* partially rescues the phenotype induced by the mutation in HSCs while having minimal effects in wild-type cells. However, CFU assays showed that the knockout of these genes did not restore erythroid differentiation, indicating that the differentiation block caused by H3-K27M likely occurs through a separate, independent mechanism.

Conclusion: H3-K27M promotes HSC expansion and disrupts erythroid differentiation by enhancing early commitment while blocking terminal maturation. Although PLAG1 and LIN28B knockout partially rescues HSC expansion, it does not restore erythroid differentiation, suggesting that these processes are driven by distinct mechanisms.

Title: Decoding Donor Variability to Optimize MiHA-Specific TCR T Cell Engineering for Post-Transplant Leukemia Immunotherapy

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Keywords: minor histocompatibility antigens, immunotherapy, leukemia, donor variability

Background: The therapeutic efficacy of allogenic stem cell transplantation (aHSCT) relies largely on a robust graft-versus-leukemia (GVL) effect. This effect is mediated by donor T cells recognizing mismatches in antigens such as minor histocompatibility antigens (MiHAs). MiHAs are HLA-restricted self-peptides derived from single nucleotide polymorphisms. We recently established a comprehensive library of 98 MiHAs preferentially expressed on leukemic cells, providing foundation for precision T cell–based immunotherapy.

Purpose of the study: We recently demonstrated in a Phase I clinical the safety and feasibility of infusing ex vivo MiHA-specific T cells. However, clinical responses were limited due to low the frequencies of antigen-specific T cells and prolonged culture times leading to T cell exhaustion. Methods: To investigate the functional fitness of CD8 T cells obtained from different donors, we will transduce primary CD8 T cells obtained from several donors with lentivirus expressing low and high-affinity TCRβs specific for a given MiHA. The engineered T cells will be re-stimulated with APCs pulsed with cognate MiHA, and various in vitro functions will be assessed.

Results: Our results demonstrated that engineered T cells successfully responded to malignant target cells. Notably, T cells from some donors consistently exhibited greater cytokine secretion, CD107a expression, and cytotoxicity compared to other donors. Understanding the basis of this functional heterogeneity is critical to ensure reproducible manufacturing and consistent therapeutic efficacy. In this study, we investigated whether inter-donor functional variability of MiHA-TCR engineered T cells is driven by intrinsic metabolic and transcriptional programs.

Interestingly, enhanced metabolic fitness correlated with strong anti-MiHA reactivity of the engineered T cells. Elite donors also displayed enhanced glycolysis activity. This could be explained by the engineering platform used for the cells. Indeed, conventional viral transduction results in variable TCR expression on engineered cells, as we observed differences in transduction efficiency due to uncontrolled integration into random genomic loci. To achieve precise integration and eliminate the variability arising from viral transduction, we will use CRISPR/Cas9-mediated TCR knock-in to compare inter-donor variation.

Conclusion: By systematically dissecting donor-related and methodological sources of variability, this work will help identifying biomarkers of high-performing donors and define the most robust engineering strategy. The outcome will enable rational design of next-generation MiHA-specific TCR therapies with enhanced potency, reproducibility, and translational potential across hematological malignancies.

Title: Characterization of Stromal Cell Mediated Drug Resistance in Acute Myeloid Leukemia

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Keywords: Acute Myeloid Leukemia, Drug Resistance, Microenvironment, Co-culture

Background information: Acute myeloid leukemia is a heterogeneous disease that encompasses about one fifth of pediatric cases of leukemia. However, the survival of this disease remains poor at ~60% in patients aged 0-14 largely due to drug resistance and relapse. Drug resistance can be due to intrinsic properties of the AML cells or extrinsic through interactions with the microenvironment in the bone marrow. Importantly, the precise AML-microenvironment interactions leading to drug resistance are underexplored as the majority of those that are known are co-opted from normal hematopoiesis. Furthermore, no inhibitor developed for these interactions has been approved for clinical use.

Purpose of the study: Our primary goal is to elucidate AML-niche interactions and mechanisms that mediate drug resistance towards common chemotherapeutics using in vitro co-culture with HS-5, a human immortalized bone marrow mesenchymal stromal cell line.

Methods: First, we adapted the HS-5 cell line from DMEM growth media to those recommended for the AML cell lines used in this study: RPMI 1640 for THP-1, NOMO-1, and ML-2 and IMDM for SHI-1. We performed RNA-seq on the adapted cells and the parental cell line to study transcriptional changes that may have occurred during the adaption process.

We then performed annexin V-propidium iodide (PI) co-staining and flow cytometry to measure AML apoptosis after cytarabine exposure to determine if our adapted cells show the same published drug resistance phenotype in direct and indirect co-culture with a transwell insert. Also using flow cytometry, we performed volumetric counting to study cell proliferation and used PI to measure the cell cycle by DNA content. Finally, to study the effects of HS-5 and cytarabine exposure, we performed RNA-seq on THP-1 cells in monoculture and co-culture with and without cytarabine exposure.

Results: For our adapted HS-5 cells, there were only 5 differentially expressed genes in the RPMI 1640 and IMDM cultured cells. Using these cells and the 4 AML cell lines, we were then able to reproduce the cytarabine drug resistance effect. However, the enhanced chemo-protection is only observed for THP-1 and ML-2 co-cultures. Additionally, we observed a reduced proliferation effect in both the AML cell lines (THP-1 and NOMO-1) and HS-5 along with alterations to the cell cycle. Importantly, the changes in the cell cycle phases differ between the different AML cell lines. For example, we observed an increase in the G0/G1 phase for THP-1 but not ML-2 where there was a decrease in the S phase. Interestingly, our THP-1 RNA-seq results revealed that the most transcriptional changes occur with HS-5 exposure and not with the drug treatment.

Conclusion: In conclusion, while we observed a global reduction in apoptosis in our HS-5 co-culture model in all four AML cell lines, the subtle differences along with the cell cycle differences observed indicate that these cell lines react differently to the presence of HS-5. Ultimately these differences and this work will improve our understanding of microenvironment-mediated drug resistance in AML.

Title: Combined Impact of HLA Alleles and Cytokine Gene Polymorphisms on Acute GVHD Risk in Pediatric HSCT

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Keywords: Acute lymphoblastic leukemia, Pharmacogenomics, Genetic variants, Allogeneic hematopoietic stem cell transplantation, Graft-versus-host disease

Background: Graft-versus-host disease (GVHD) remains a major complication of allogeneic hematopoietic stem cell transplantation (HSCT). While human leukocyte antigen (HLA) compatibility is a key determinant, increasing evidence suggests that both HLA alleles and

non-HLA immune gene polymorphisms, particularly cytokine-related single nucleotide polymorphisms (SNPs), contribute to GVHD risk.

Purpose: This study aimed to evaluate the association between patients' HLA alleles and acute GVHD (aGVHD) risk, and to validate previously reported cytokine-related SNPs in a pediatric HSCT population, analyzing their combined effect on aGVHD.

Methods: Pediatric HSCT recipients were recruited and divided into discovery (n=87) and replication (n=154) cohorts from institutional and multicenter biobank sources. Genotyping was performed using whole exome sequencing (WES) for the discovery cohort and allele-specific PCR for replication. Fifty-eight HLA alleles with carrier frequency >5% and 25 SNPs across 12 cytokine genes were analyzed for associations with aGVHD grades II–IV, both independently and in combination.

Results: Two HLA alleles, *HLA-DRB1*07:01* and *HLA-B*15:01*, were significantly associated with increased aGVHD risk (p=0.004 and p=0.009), particularly in HLA-matched unrelated donor settings. Carriers of these alleles were also more likely to present non-permissive *HLA-DPB1* mismatches. Additionally, two cytokine SNPs, *IL1B* rs1143627 and *IL17A* rs8193036, were significantly associated with higher aGVHD risk (p=0.019 and p<0.001), with stronger effects observed in HLA-matched pairs. Notably, individuals carrying one or both cytokine risk alleles in combination with *HLA-B*15:01* exhibited a markedly elevated risk (HR: 2.14, CI: 1.41–3.25, $p=6\times10^{-6}$).

Conclusion: These findings highlight the combined contribution of HLA alleles and non-HLA cytokine genetic variants in aGVHD pathogenesis. Integrating cytokine SNP profiling with HLA typing may improve donor selection and support personalized strategies for aGVHD risk assessment and prophylaxis, particularly in HLA-matched unrelated donor transplantation.

Title: Structure and function analysis of PARP inhibitors for the development of next-generation leukemia therapeutics

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Keywords: PARP1, DNA damage, leukemia, inhibitors, structural biology

Background: Poly(ADP-ribose) polymerase 1 (PARP1) plays a key role in the DNA damage response. Upon binding to DNA breaks, PARP1 modifies itself and other proteins with poly(ADP-ribose) (PAR), triggering the recruitment of DNA repair factors. Cancers carrying specific deficiencies in DNA repair genes rely on PARP1 for genome preservation, which is targeted in the clinic with PARP inhibitors (PARPi). Currently, four

PARPi are approved for the treatment of solid tumours, while clinical trials have explored PARPi in leukemia. PARPi vary in tumour-killing efficiency in part based on their allosteric impact on PARP1 binding to DNA. Type I PARPi strengthen PARP1 interaction with DNA and are underrepresented in the clinic, while type II does not impact DNA binding, and type III weaken this interaction. Our recent work showed that chemically converting a type III PARPi to type I enhances PARP1 retention on DNA and cancer cell killing, revealing a promising strategy still untested in leukemia.

Purpose: We propose to elucidate the allosteric effects of new generation PARPi on the structure of full-length PARP1 bound to DNA, biochemically characterize novel PARPi, and assess their efficiency at killing leukemia cells.

Methods: We employ fluorescence polarization assays to assess PARP1 binding to DNA as a function of PARPi. Novel PARPi are provided by collaborators. Atomic-level insights on DNA-bound, full-length PARP1 in the presence of PARPi are monitored by cryo-electron microscopy (cryo-EM). The activity of new PARPi in leukemia is assessed with KASUMI-1 and THP-1 cell lines. KASUMI-1 harbours the AML1-ETO fusion oncogene, which is present in 10-20% childhood AML and leads to several malfunctioning DNA repair proteins, resulting in PARPi monotherapy sensitivity. THP-1 cells bear the MLL-AF9 translocation, which is found in 25% *de novo* AML in children and renders cells less sensitive to PARPi. Trypan blue exclusion assays will be used to monitor cell viability and validated by the CellTiter-Glo® 2.0 Cell Viability Assay. PARP1 retention on DNA at the cellular level and catalytic activity inhibition will be measured, respectively, using chromatin fractionation assays and immunoblotting as a function of PARPi.

Preliminary Results: Our recent cryo-EM studies with full-length PARP1 bound to damaged DNA, protein partners, and a type I PARPi revealed an unanticipated flexibility of PARP1's catalytic domain, suggesting localized and acute PAR production at an expanded radius relative to the DNA damage site. Although the tested type I PARPi showed no immediate superiority to approved therapeutics in initial screens with THP-1 cells, limited solubility hindered more rigorous cell-based assays. Current research is directed toward improving these profiles to fully characterize their biological potential.

Conclusions: This work will be the first to evaluate novel PARPi in leukemia cells and will provide invaluable structural and biophysical insights that will be the basis for future clinical studies. In assessing different types of PARPi and their effectiveness in killing leukemia cells, the results of our study may serve as benchmarks for tailored drug development in the future.

Title: A novel role of the transcription factor TCF-1 in the regulation of the Src-family kinase p56LCK: Implications in T-cell biology and T-cell Acute Lymphoblastic Leukemia.

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Keywords: TCF-1, p56LCK, tyrosine phosphorylation, T cell, T-cell Acute Lymphoblastic Leukemia.

Background information: T-cell-based therapies rely on precise regulation of activation, differentiation, and survival of T-cells to achieve effective and durable anti-tumor responses. However, strong TCR signaling, which relies on p56LCK activity, can drive cytokine release syndrome, whereas insufficient signaling can limit cytotoxicity. TCF-1 is a key transcriptional factor that regulates T cell development, maintains T cell stemness and shapes effector potential. Thus, TCF-1 represents a good candidate to tune effector functions, and a better understanding of it may uncover strategies to enhance therapeutic efficacy and/or to reduce treatment-related toxicity. Yet, the TCF-1isoform-specific mechanisms through which it modulates T-cell signaling and functionality remain largely unexplored. These questions are particularly relevant in the context of primary and malignant T-cell, where TCF-1 could regulate survival pathways and drug responsiveness in T-cell acute lymphoblastic leukemia (T-ALL) patients.

Purpose of the study: This study aimed to determine how TCF-1 isoforms could modulate the TCR signaling pathway, effector differentiation, cytotoxic function, and survival in both primary $CD8^+$ T cells and malignant T-cell models.

Methods: We overexpressed individual TCF1 isoforms (p45 and p33) in primary murine OT-1 $CD8^+$ T cells and human acute T-cell lymphoblastic leukemia Jurkat cells to assess their effects on TCRinduced signaling and functional responses. Proximal signaling events were evaluated through phosphoflow cytometry. Effector differentiation was quantified by CD25, Granzyme B, and cytokine production following TCR engagement. Cytotoxicity assays were performed against Tcell lymphoma and melanoma targets using flow cytometry and fluorescence microscopy. Cell survival, proliferation, and drug sensitivity were assessed in vitro using viability dyes, cellcounting assays, and doxorubicininduced division arrest.

Results: Our findings show that p45 and p33 TCF-1 isoforms enhance TCR-related signaling events, paradoxically accompanied by reduced effector differentiation and cytokine production upon TCR stimulation.

Despite this reduced effector profile, preliminary assays revealed that TCF-1-overexpressing $CD8^+$ T cells retained similar capacity to kill both T-cell lymphoma and melanoma targets in vitro. Because TCF-1 also promoted survival in primary $CD8^+$ T cells, we investigated whether similar effects occur in malignant T cells. Preliminary data indicate that neither TCF-1 isoform altered proliferation or total cell numbers in Jurkat cells, but both isoforms reduced sensitivity to doxorubicin-induced inhibition of cell division.

Conclusion: Our results reveal that TCF-1 isoforms can enhance TCR-proximal signaling in CD8+ T cells, while simultaneously restraining effector differentiation and maintaining cytotoxic capacity against tumor targets. This rewiring could preserve cytotoxic function and mitigate cytokine release syndrome caused by engineered T cell therapy. TCF-1 promotes survival in primary $CD8^+$ T cells and cell division in doxorubicin-treated malignant T cells, suggesting its potential as a biomarker of drug-resistance.

Title: Diversity in the Mechanisms of Action of Anthracyclines

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Keywords: Anthracyclines, Aclarubicin, CRISPR-Cas9, Leukemia, Mechanism of Action

Background information: Topoisomerases are essential enzymes that relieve DNA torsional stress during replication, transcription, and chromatin remodeling, making them key targets in cancer therapy. Anthracyclines such as doxorubicin and daunorubicin are widely used chemotherapeutic agents that function primarily as topoisomerase II poisons. Aclarubicin, another anthracycline used in leukemia treatment in Asia, has been described as a dual topoisomerase I and II inhibitor. Notably, Aclarubicin exhibits reduced cardiotoxicity and lacks cross-resistance with other anthracyclines. However, its precise molecular mechanisms of action remain poorly understood.

Purpose of the study: This study aims to characterize and compare the molecular mechanisms of Aclarubicin and other anthracyclines, with a focus on identifying pathways underlying their differential cytotoxic effects.

Methods: Genome-wide CRISPR-Cas9 chemogenomic screens were performed in NALM-6 pre-B acute lymphoblastic leukemia cells to identify genes involved in drug sensitivity and resistance. Comparative analyses between Aclarubicin and doxorubicin profiles were conducted.

Functional validation included genetic perturbation of candidate pathways, assessment of apoptosis, and evaluation of transcriptional activity using Northern blot assays to measure RNA polymerase I activity.

Results: CRISPR-Cas9 screens indicated minimal overlap between Acla and Dox in sensitivity and resistance genes. Interestingly, Acla cytotoxicity was not rescued by loss of TOP2A/B or DNA repair genes, indicating a mechanism independent of topoisomerase II poisoning. Instead, loss of genes involved in the nucleolar stress response and apoptosis caused resistance, pointing toward a distinct mechanism of action leading to p53 stabilization and activation of apoptotic programs. Moreover, Acla suppressed RNA polymerase I transcription in Northern assays, supporting inhibition of ribosome biogenesis and of global protein translation as a key driver of its cytotoxicity.

Conclusion: Our findings highlight the distinct cellular responses to Acla compared to other anthracyclines, supporting its potential as an effective therapeutic tool in relapsed/refractory leukemia cases. Understanding Acla's mechanism of action will optimize its clinical use and guide the development of improved analogs with reduced toxicity. Expanding this study to additional leukemia models and patient-derived samples will further help identify companion biomarkers predictive of response.

Title: Engineering TacrolimusResistant Regulatory T Cells to Enhance GVHD Control Under Pharmacological Immunosuppression

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Keywords: GVHD; Regulatory T cells (Treg); Tacrolimus; FKBP12; CRISPR-Cas9

Background information: Allogeneic hematopoietic stem cell transplantation (HSCT) remains a potentially curative option for severe leukemia and lymphoma, but its success can be limited by graft-versus-host disease (GVHD), a major source of morbidity and mortality. Regulatory T cells (Tregs) are highly attractive for adoptive immunotherapy because they can suppress harmful alloreactivity while preserving beneficial transplant effects. However, tacrolimus, one of the most used agents for GVHD prophylaxis, may also inhibit Treg expansion by blocking FKBP12-dependent calcineurin-NFAT signaling. This creates a therapeutic paradox in which the drug used to control GVHD may also reduce the

efficacy of Treg-based therapy. These observations provide a strong rationale for engineering tacrolimus-resistant Tregs able to remain functional under tacrolimus treatment.

Purpose of the study: This study aimed to determine whether CRISPR-Cas9-mediated deletion of FKBP12 could render human ex vivo-expanded Tregs resistant to tacrolimus while preserving their phenotype, stability, and suppressive function. The broader translational objective was to support a strategy in which engineered Tregs remain functional despite ongoing tacrolimus exposure in a GVHD setting.

Methods: Human Tregs from healthy donors were isolated from peripheral blood mononuclear cells (PBMCs), expanded ex vivo, and edited using CRISPR-Cas9 to delete FKBP12. After allowing time for residual FKBP12 protein degradation, edited and control Tregs were cultured with tacrolimus (10 ng/mL) under varying IL-2 concentrations (100-500 IU/ml). Proliferation was evaluated by cell counts, proliferation dye dilution, and Ki-67 expression, while NFAT1 nuclear translocation was assessed as a mechanistic readout. Flow cytometry, inflammatory challenge assays, bulk RNA sequencing, and *in vitro* suppression assays were performed to evaluate lineage stability, phenotype, migratory profile, transcriptomic integrity, and suppressive capacity.

Results: Tacrolimus significantly impaired proliferation of control Tregs, even under high IL-2 conditions, whereas FKBP12-knockout Tregs maintained robust proliferation, preserved Ki-67 expression, and retained NFAT1 nuclear translocation despite tacrolimus exposure. Gene editing did not compromise classic Treg markers, including FOXP3 and Helios, and it did not substantially affect activation, memory, or homing-related phenotypes. Transcriptomic analysis showed minimal differences between edited and control Tregs, and suppressive assays confirmed that FKBP12-KO Tregs remained functionally *in vitro*. These findings indicate that tacrolimus resistance can be introduced without loss of Treg identity.

Conclusion: This study provides a proof of concept for a next-generation Treg therapy compatible with tacrolimus-based management. Tacrolimus-resistant Tregs could preserve immune regulation in HSCT recipients while tacrolimus continues to suppress pathogenic effector T cells, thereby improving GVHD control without requiring broader combination immunosuppression. This approach supports the development of a more targeted and potentially safer cellular therapy platform for patients undergoing HSCT.

Title: Comparative translatomic and proteomic profiling of three eIF4A inhibitors reveals convergent AML dependencies and adaptive responses

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Keywords: Acute myeloid leukemia; eIF4A; translatomics; proteomics; drug resistance

Background: Acute myeloid leukemia (AML) frequently relapses through adaptive remodeling of oncogenic signaling, metabolism, and apoptotic control. Many of these resistance-associated programs converge on cap-dependent mRNA translation, which is controlled by the eIF4F complex. eIF4A, the RNA helicase subunit of eIF4F, is particularly attractive therapeutically because it is required for efficient translation of transcripts encoding growth, survival, and metabolic regulators. Selective eIF4A inhibitors show potent antitumor activity in preclinical models; importantly, zotatifin (eFT226) has advanced to phase 1/2 clinical testing, while the second-generation rocaglate MG-002 is orally bioavailable and shows favorable in vivo properties, supporting the therapeutic tractability of this class. However, their translatomic effects have not been directly compared in AML, and the extent to which early translational changes are reflected at the proteome level remains unclear.

Purpose: We aimed to define the common and compound-specific molecular response to three eIF4A inhibitors (eIF4Ai; CR-1-31-B, MG-002, and zotatifin) in AML, and to identify AML-relevant pathways and candidate biomarkers revealed by integrated translatomic and proteomic profiling.

Methods: MOLM-14 AML cells (*KMT2A:MLLT3*, *FLT3*^{ITD}) were treated with DMSO, CR-1-31-B, MG-002, or zotatifin. Drug doses were selected using OP-puromycin incorporation assays to achieve comparable inhibition of protein synthesis across eIF4Ai. Translatome (1h treatment) and proteome (24h) changes were measured, coupled with Hallmark/Reactome enrichment and cross-omics integration.

Results: Comparative translatomics revealed a substantial shared response to eIF4Ai, with 628 translation-efficiency genes altered by all three compounds; CR-1-31-B and MG-002 produced broader

effects than zotatifin. Shared translationally suppressed programs included MYC/E2F-driven proliferation and cell-cycle control, PI3K/AKT/mTOR and RAS/MAPK/RTK signaling, and protein trafficking/secretion. Proteomics identified more than 1,400 significantly altered genes for each drug, including 961 shared across all three inhibitors, and showed recurrent depletion of E2F, G2M, PI3K/AKT/mTOR, KRAS-associated, and cell-cycle programs. At the same time, oxidative phosphorylation, unfolded protein response, and mitochondrial translation signatures were increased, consistent with adaptive stress remodeling. Cross-omics integration showed significant overlap between translatomics and proteomics for each inhibitor, with 77–81% of overlapping genes changing in the same direction.

Conclusion: These data identify a conserved AML response to eIF4A inhibition that is shared across three distinct inhibitors and has not previously been defined in this disease at the translatome level. The earliest and most concordant effects are suppression of proliferative and signaling outputs that support AML maintenance. This integrated dataset highlights candidate biomarkers and combination targets for future studies and provides a framework to understand how eIF4A inhibition rewires leukemic cells across multiple regulatory layers.

Title: Unlocking Therapeutic Vulnerabilities and Drug Resistance Mechanisms in Leukemia with CRISPR-Based Screens

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Keywords: Leukemia, CRISPR Activation, Genetic Engineering, Drug Resistance

Background: Leukemia is a cancer of hematopoietic stem cells in the bone marrow. It is classified as lymphoid or myeloid, and as acute (rapid onset) or chronic (slow progression). While AML, CML, ALL, and CLL are distinct, comparative studies highlight shared and unique features. Identifying key pathways across subtypes may yield improved treatments. Advances in CRISPR technology enable deeper study of leukemia biology and therapeutic targets. CRISPR knockout screens have been performed across these cancer subtypes by the cancer dependency group to identify cell line specific susceptibilities. An alternate form of CRISPR perturbation is CRISPR activation (CRISPRa). This

technology utilizes an enzymatically inactive form of CAS9 fused to a VP46 transcriptional enhancer protein, in addition to a fusion of effectors called MPH, for potent and specific transcription of user-defined genes.

Purpose: Our lab utilizes CRISPRa to increase the endogenous expression of genes, enabling us to identify the function of a given gene on cancer cell growth. We hypothesize that genome-wide CRISPRa screening will uncover subtype-specific genetic dependencies and drug resistance mechanisms, offering novel therapeutic insights.

Methods: We engineered leukemia cell lines by integrating a self-selecting CRISPRa plasmid carrying a blasticidin resistance gene using a Piggybac transposase system. Cells were transduced with our whole-genome CRISPRa library with puromycin selection to enrich for sgRNA-expressing cells. Post-transduction, cells were harvested at various timepoints. These cells were sequenced to track sgRNA abundance and identify growth-modulating genes. We quantified the effect of each gene through measuring the LOG2 of the fold change of each sgRNA over time. We normalized the fold change for each cell line to the number of cell doublings between our time points.

Results: We performed whole genome CRISPRa screens in three cell lines; K562 (CML), THP-1 (AML), and Jurkat (T-ALL). In CRISPR knockout screens, more than 90% of hits are shared across cell lines. With CRISPRa, we have been able to identify more than 300 unique genes that modify cell growth. Of these genes, 49 were detrimental to cell growth across all three subtypes of leukemia but each cell line had over 200 unique genes that suppressed growth. The genes identified in our screens were distinct from those observed in the knockout screens, showing more cell-specific enriched pathways like cell differentiation an immune activation. To validate the screen results, we tested individual sgRNAs for TNFRSF10A and TNFRSF10B in K562 cells and observed an increase in protein expression and suppression of cell growth.

Conclusions: These CRISPRa screens reveal genes that, when activated, influence leukemia cell growth. We were able to observe distinct gene hits across each of our leukemia subtypes, highlighting unique susceptibilities in each cell line. We plan to perform Perturb-seq, a method to identify each CRISPR perturbation's impact on the cell's transcriptome through single cell RNA-seq using a selective library of sgRNAs. This will clarity on how activation of each gene is modifying the state of the cell. These findings support discovery of new therapeutic targets and enhance understanding of leukemia subtype biology, guiding future personalized treatment strategies.

Title: Plasma proteome characterization of Acute Megakaryoblastic Leukemia xenograft models

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Background information: Acute megakaryoblastic leukemia (AMKL) is a rare subtype of acute myeloid leukemia (AML) that is more common in children, accounting for 4–15% of pediatric AML cases, particularly in those under 3 years of age. It frequently involves recurrent, mutually exclusive gene fusions such as *CBFA2T3::GLIS2* (CG2), *NUP98::KDM5A* (N5A), *and NUP98::BPTF* (NTF), and is associated with poor prognosis despite intensive therapy, highlighting the urgent need for targeted treatments. Plasma is a rich source of biomolecules, including proteins that reflect the physiological and pathological state of the body. Because of their central roles in biological processes, proteins represent an attractive source of biomarkers and have fueled the rapid expansion of plasma proteome profiling. This approach is emerging as a non-invasive and promising strategy for disease diagnosis, monitoring, and identification of therapeutic targets in leukemia.

Purpose: To identify AMKL subtype-specific signatures and uncover therapeutic vulnerabilities by profiling the plasma proteome of xenograft models.

Methods: An antibody-based proximity extension assay (PEA; Olink Reveal) was performed on plasma from AMKL mouse xenograft models (CG2, N5A, and NTF) established in our laboratory. Briefly, 1×10^6 leukemic cells (CG2 n=42, N5A n=5, NTF n=5) or normal hematopoietic stem and progenitor cells (HSPCs, $CD34^+$) from cord blood (n=5) were injected into immunodeficient mice. Mice developed leukemia with variable latency and were euthanized based on blood infiltration and clinical condition. At endpoint, blood was collected in EDTA tubes, and plasma was isolated after centrifugation. Plasma from HSPC $CD34^+$ transplanted mice was used as a normal human hematopoietic cell reconstitution control, while non-transplanted NSG/SGM3 mice was used to exclude proteins not reliably classified as human or mouse. 1,084 proteins were evaluated, and normalized protein expression (NPX) values were used for differential expression analysis. Plasma from an N5A patient was included and compared to cord blood plasma. Selected hits were validated using ELISA. Additional plasma proteomics approaches are currently being optimized.

Results: Patient-derived AMKL xenografts showed robust engraftment, with human CD45+ infiltration in bone marrow (CG2: 80%, N5A:

45%, NTF: 20%) and spleen (CG2: 81%, N5A: 75%, NTF: 50%), supporting human leukemic reconstitution and recapitulation of patient features. Plasma proteomic profiling identified 979 human proteins after excluding those enriched in background controls. Each model displayed a distinct plasma signature compared to controls and other AMKL models. The CG2 group showed 150 differentially expressed proteins, including upregulation of FGF23, ACE2, and PGF. PGF is associated with proliferation, migration, and angiogenesis, suggesting an aggressive phenotype. In N5A plasma, 65 proteins were differentially expressed; FASLG was increased and linked to immune evasion and chemotherapy resistance and validated by ELISA. NTF mice showed increased TNF-α, IL7, and IL4R. TNF-α upregulation was independently confirmed using Luminex, highlighting inflammatory signaling in NTF models.

Conclusions: The identification of plasma analytes in AMKL xenograft models may help define trackable subtype-specific targets, improve our understanding of drug mechanisms of action, and ultimately guide the development of more effective and less invasive therapeutic strategies for high-risk pediatric AMKL.

Title: Targeting Transcriptional and Translational Programs in AML with KDM4A Inhibitors and Rocaglates

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Keywords: Pediatric AML, KDM4A, Cellular senescence, Rocaglates, mRNA translation

Background information: Acute Myeloid Leukemia (AML) is a heterogeneous blood cancer marked by uncontrolled proliferation of immature myeloid blasts from hematopoietic stem/progenitor cells. Therapy resistance in AML remains a major clinical obstacle. Key resistance mechanisms include oncogenic gene transcription and deregulated protein synthesis, driven by epigenetic modifications and translation initiation. Epigenetics alters gene expression without changing the DNA sequence, primarily via modifications of the chromatin state. Lysine demethylases (KDMs) like KDM4A-C remove methyl groups from histones to modulate chromatin structure. KDM4A is overexpressed in pediatric AML with the MLL-AF9 translocation (KMT2A-MLLT3 fusion), driving cell proliferation. Similarly, translation initiation, controlled by the eIF4F complex and its ATP-dependent helicase eIF4A, is crucial for chemoresistance, with AML cells expressing elevated eIF4A1 levels.

Purpose of the study: We propose that dual targeting of KDM4A and eIF4A in pediatric AML will eradicate malignant cells and limit residual disease persistence.

Methods: We evaluated whether a sequential therapeutic strategy consisting of epigenetic inhibition (QC6352 KDM4A inhibitor) followed by translation inhibition (CR-1-31B eIF4A inhibitor) enhances the elimination of resistant and dormant leukemic cells. In vitro studies were performed using the AML cell lines THP-1, Mono-Mac-1, and MOLM-14 (all bearing the MLL-AF9 fusion). High-throughput sequencing of the translatome will be used to define the translational signature of cells persisting after epigenetic inhibitor treatment and to identify features associated with sensitivity to subsequent translation inhibitor-mediated elimination.

Results: In vitro, THP1 and MOLM14 cells demonstrated increased sensitivity to sequential treatment with QC6352 and CR-1-31-B. Epigenetic inhibitor treatment reshaped the translational landscape of resistant cells, while subsequent translation inhibition reduced the synthesis of key pro-survival proteins. By identifying genes required for the persistence of resistant cells, we uncovered potential vulnerabilities that may be therapeutically targeted at the translational level to overcome resistance mechanisms. Next steps: In vivo, we expect that combining QC6352-mediated KDM4A inhibition with rocaglate-mediated eIF4A inhibition will leverage the low toxicity observed with each monotherapy while targeting key adaptive mechanisms in malignant cells. Additionally, the senolytic properties of rocaglates may eliminate treatment-induced senescent cells that could otherwise foster a metastasis-promoting niche and elevate relapse risk.

Conclusion: Our results indicate that a sequential treatment targeting both the epigenetic regulation of oncogenic circuits and the aberrant synthesis of survival proteins in AML cells effectively eliminates cancer cells and reduces residual malignancy. We propose that inhibiting KDM4A with QC6352 impairs neoplastic cell growth by inducing senescence state like, relying on an anti-apoptotic protein expression. Since eIF4A inhibitors disrupt the translation of key transcripts, including anti-apoptotic BCL2-family members, the senescence induced by KDM4A inhibition heightens sensitivity to rocaglates. Consequently, subsequent rocaglate treatment eliminates these senescent cells by preventing the synthesis of survival proteins.