Cole Foundation

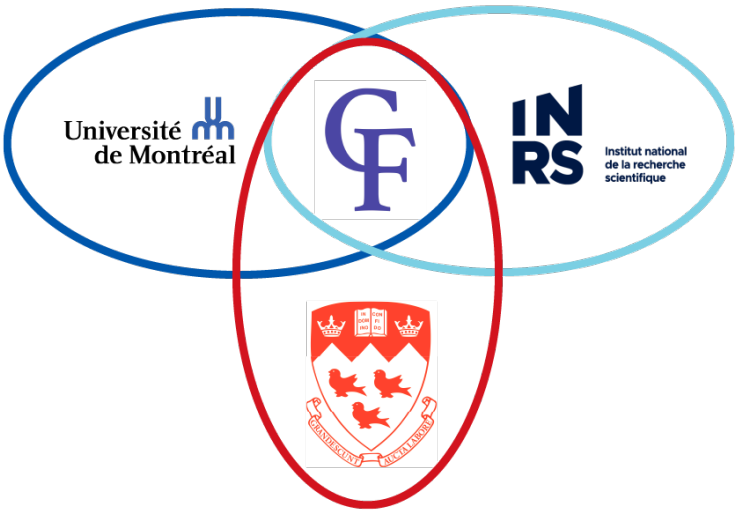
«Research Celebration Day»

19th Year

Journée «Célébrons la recherche»

de la Fondation Cole

19e Année



The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD programmes 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 2025 Fellows, the Fellowship programme has supported more than 168 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.

Over $1.5 million has been committed to this programme. 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 2025, le programme a appuyé plus de 230 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. Plus de 14 millions $ y ont été consacrés.

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

"RESEARCH CELEBRATION DAY"

in support of pediatric and young adult leukemia and lymphoma research

Friday, May 2nd, 2025

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 Maureen McKeague** – Canada Research Chair in Functional Oligonucleotides, McGill University
  "Manipulating parthanatos to improve AML treatment outcomes"
- **Dr Benjamin Haley** – Centre de recherche de l'Hôpital Maisonneuve-Rosemont
  "Scalable Functional Genomics to Decode Mechanisms of Leukemic Cell Growth and Differentiation"
- **Dr Julianna Blagih** – Centre de recherche de l'Hôpital Maisonneuve-Rosemont
  "Over-Sweetening the Pot: Mixing Sucralose into T-ALL Treatments"

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

Keynote speaker: Dr Nirali N. Shah, MD, MHSc

NIH Lasker Clinical Research Scholar Head, Hematologic Malignancies Section Pediatric Oncology Branch National Cancer Institute

"CAR T-cell therapy in adolescents and young adults: 2025 and beyond!"

Dr. Shah is a physician scientist who serves as an NIH Lasker Clinical Scholar and Head of the Hematologic Malignancies Section of the Pediatric Oncology Branch, the goal of which is to implement early-phase immunotherapeutic approaches for high-risk hematologic malignancies. Her research focuses on the development of targeted immunotherapy approaches to treat high-risk blood cancers, such as leukemia and lymphoma, in children, adolescents, and young adults. She is also interested in the prevention and treatment of relapsed disease after allogeneic hematopoietic stem cell transplantation. Her clinical trials focus on advancing and improving chimeric antigen receptor (CAR) T-cell based therapies and other antibody-based therapies to target surface proteins found on leukemia cells to improve outcomes for patients with blood cancers that do not respond to chemotherapy. Her research aims to improve response while reducing the toxicities and late effects of these therapies.

A reception will follow.

Address:
Université de Montréal
Pavillon Roger-Gaudry, Hall d'honneur
2900, boulevard Édouard-Montpetit, Montréal, Québec

McGill University

■ **Dr. Emily Drake,** Post-PhD program

Supervisors: Dr. Argerie Tsimicalis and Dr. Michel Duval

Title: Knowledge Mobilization Needs Assessment of the Adolescent and Young Adult Cancer Community

Abstract: Our study will describe and prioritize the knowledge mobilization needs of the adolescent and young adult (AYA) cancer community. Employing a community-based, participatory, concurrent triangulated, mixed methods research design, guided by knowledge mobilization frameworks, this study will be the first of its kind to map the knowledge mobilization needs and priorities of the AYA cancer community.

■ **Ekkanat Jiramongkolsiri,** PhD program

Supervisor: Dr. Sidong Huang

Title: Deciphering and Exploiting the Role of eIF2A in Modulating Chemotherapy Response in Lymphoma

Abstract: Chemoresistance remains a major challenge in cancer treatment. We have found that suppressing eukaryotic initiation factor (eIF) 2A resensitizes lymphoma cells to front-line chemotherapeutic drugs. This study aims to uncover its mechanism of action using CRISPR/Cas9, multi-omics approaches, and functional genetic screening.

■ **Linda Pallotto,** PhD program

Supervisor: Dr. Chantal Autexier

Title: Regulation of telomere length homeostasis by a unique telomerase domain

Abstract: Telomere length dysregulation contributes to acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) development. Our project aims to better understand how the telomerase enzyme regulates telomere length, which we will achieve by investigating the activity of several mutants.

Université de Montréal

- **Javier Anleu Alegria,** PhD program

  Supervisors: Dr. Trang Hoang and Dr. Pierre Thibault

  Title: Vulnerabilities of T-cell acute lymphoblastic leukemia revealed by immunopeptidomics

  Abstract: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive pediatric cancer requiring intensive chemotherapy treatment, with severe and long-lasting side effects on patients. Our goal is to identify tumor-specific antigens for immunotherapy to complement drug treatment, reducing side effects, and improving patients' quality of life and long-term survival.

- **Elsa Berliocchi,** PhD program

  Supervisor: Dr. Philippe Roux

  Title: Proteomic analysis of AMKL fusion proteins for therapeutic target identification

  Abstract: Using proteomics, we aim to identify proteins associating with the CBFA2T3::GLIS2 fusion, a key driver of pediatric Acute Megakaryoblastic Leukemia (AMKL). These proteins may reveal novel mechanisms of leukemogenesis and serve as potential therapeutic targets in this rare and aggressive leukemia.

- **Anshul Budhraja,** PhD program

  Supervisors: Dr. Vincent Lavallée, Dr. Martin A. Smith

  Title: Leveraging Long-Read Single-Cell RNA Sequencing for Improved Fusion Transcript and Isoform Detection

  Abstract: My research aims to better understand gene regulatory changes underlying leukemia by identifying AML-specific isoforms. To this end, I will use long-read single-cell sequencing. These insights will enhance molecular profiling of leukemia, aiding in early diagnosis, disease classification, and the development of targeted therapies.

- **Laurence Côté,** PhD program

  Supervisor: Dr. Julie Lessard

  Title: Targeting the SMARCD1-p53 Axis in Pre-B Acute Lymphoblastic Leukemia

  Abstract: This project investigates the role of the SMARCD1-WTp53 axis in B-cell acute lymphoblastic leukemia (B-ALL). We aim to define its contribution to leukemogenesis and metabolic reprogramming and assess the therapeutic potential of disrupting this interaction to impair leukemia progression using genetic models and protein-protein interaction inhibitors.

- **Juliette Durocher,** PhD program

  Supervisor: Dr. Marissa Paige Rashkovan

  Title: Disturbing mitochondrial metabolism as a potential therapeutic strategy in ETP-ALL

  Abstract: Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) is a high-risk subtype of T-ALL with a more dismal prognosis due to high incidence of relapse. My project will investigate the role of the mitochondria in ETP-ALL, and the therapeutic relevance of targeting essential mitochondrial genes as a potential new treatment strategy.

- **Charlotte Gélinas-Gagné,** PhD program

  Supervisors: Dr. Serge Sultan and Dr. Karine Bilodeau

  Title: Améliorer le bien-être des adolescent.es et jeunes adultes survivant.es de cancers pédiatriques hématologiques : Une étude approfondie sur les comportements de santé et les habitudes de vie

  Abstract: Ce projet vise à explorer les habitudes de vie saines chez les adolescent.es et jeunes adultes survivant.es d'un cancer hématologique pédiatrique. Il comprend trois phases : une revue de portée pour identifier les leviers de changement dans les interventions existantes, une enquête mixte pour comprendre les facteurs motivants les comportements de santé, et le co-développement d'une intervention innovante.

- **Carlos Andres Oroya Lazo,** PhD program

  Supervisor: Dr. Christopher E. Rudd, Professor, Department of Medicine, Universite de Montreal and the Centre de recherche de l'Hôpital Maisonneuve-Rosemont (CRHMR)

  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.

  Abstract: Dysregulation of the transcription factor TCF-1 and the Src-family kinase p56LCK, essential proteins for T-cell developm Dr. ent and anti-tumor immunity, is associated with T-cell acute lymphoblastic leukemia (T-ALL). We discovered a novel link between TCF-1 and p56LCK and aim to investigate this connection's significance in T-cell function and T-ALL biology.

- **Belal Osman,** PhD program

  Supervisor: Dr. Sylvie Mader

  Title: Causes of Diversity in the Mechanisms of Action of Anthracyclines

  Abstract: While treatment with most anthracyclines leads to significant cardiotoxicity and is limited by resistance, aclarubicin exhibits reduced

side effects and lack of cross-resistance. Our group is using CRISPR-Cas9 chemogenomic screens in leukemia models to identify genes involved in aclarubicin sensitivity/resistance to support its therapeutic use in pediatric and adult AML.

- **Christopher Joseph Requejo Cier,** PhD program

  Supervisor: Dr. Caroline Lamarche

  Title: Optimization of Tregs-based immunotherapy through genetic modifications

  Abstract: Adoptive immunotherapy utilizing regulatory T lymphocytes (Tregs) offers a groundbreaking solution for addressing GVHD. Through advanced genetic engineering with CRISPR technology, this research aims to overcome two significant barriers: enhancing Tregs' resistance to immunosuppressants like tacrolimus by targeting the FKBP12 gene and preventing cellular exhaustion by modulating

- **Anavasadat Sadr Hashemi Nejad,** PhD program

  Supervisors: Dr. Laura Hulea and Dr. François Mercier

  Title: Targeting eIF4A to overcome therapeutic resistance in Acute Myeloid Leukemia

  Abstract: The project investigates the therapeutic potential of targeting eukaryotic initiation factor 4A (eIF4A), a key translation factor, using eIF4A inhibitors to disrupt protein synthesis and metabolic reprogramming in acute myeloid leukemia (AML), aiming to overcome resistance in high-risk and relapsed AML patients, particularly those treated with targeted therapies.

- **Jeffrey Sullivan,** PhD program

  Supervisor: Dr. Benjamin Haley

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

  Abstract: We have developed a new "gene activation" platform, which we are applying across a range of leukemic cell types to identify genes that influence cell growth and differentiation. I will integrate my results with data from the Cancer Dependency Map as a means to prioritize new drug targets.

Title: Chimeric Antigen Receptor (CAR) T Cells with Distinct Hinges for Enhanced Anti-Leukemia Activity

Authors: Dr. Milad Ahmadi Najafabadi, Dr. Fatemeh Yousefi, Dr. Xueyang Guo and Dr. Christopher E. Rudd

Affiliation: Faculté de médecine, UDM, Dépt. de microbiologie, infectiologie et immunologie, Centre de recherche et polyclinique de l'Hôpital Maisonneuve-Rosemont, IHOT

Keywords: CAR T cells, leukemia, hinge modification, tumor regression, immunotherapy

Background information: Chimeric antigen receptor (CAR) T cell therapy has emerged as a promising approach for the treatment of childhood leukemias. However, there are often limitations in efficacy and toxicity. The optimisation of CAR constructs is crucial for improving therapeutic outcomes.

Purpose: This study aimed to investigate the impact of different hinge modifications on the functionality of CD19-targeting CAR-T cells, assessing their efficacy in both in vitro and in vivo leukemia models.

Methods: We designed new CD19-specific CAR constructs with distinct hinge regions and transduced murine T cells to study cell therapy against leukemia/lymphoma. Using an improved viral transduction model, the generated CAR-T cells were evaluated for *in vitro* killing of A20 leukemia cells, a diffuse large B-cell lymphoma (DLBCL) model accounting for 10-20% of childhood non-Hodgkin lymphomas. We also examined the *in vivo* elimination of an EL4 lymphoma tumor model in mice following intraperitoneal injection. Flow cytometry analyzed tumor-infiltrating T-cells, their activation markers (CD69, TCF1, Ki67), cytokine production (TNF-α, IFN-ɣ), and exhaustion (TIM3, PD1). Further, viSNE Stochastic Neighbor Embedding utilizing AI/ML algorithms was employed. Modified cytotoxicity assays were performed to determine the tumor-killing efficiency of each CAR construct. Examination of other models of childhood acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) involving Nalm-6, HL-60 cells and other models are underway.

Results: One newly designed CAR was more effective than current clinical CARs against leukemia and lymphoma models. This was observed in our *in vitro* CAR-T cell killing assays, as well as *in vivo* models where some solid tumors were eliminated. Different CAR-T cells exhibited variability in killing efficiency, cytokine production, internalization, and in vivo tumor killing. Crucial to its successful efficacy was the concurrent

increase in activation markers and cytokine production with reduced CAR-T cell exhaustion.

Conclusion: The successful development of an *in vitro* model of tumor killing will serve as a potential platform of screening new CARs, while the strategic modification of the hinge region in CAR-T cells has demonstrated enhanced *in vitro* and *in vivo* tumor killing capabilities, linked to reduced exhaustion. These findings highlight the potential of hinge optimization in improving CAR-T cell therapy for childhood leukemia, offering a promising avenue for future clinical translation.

Title: Caractérisation moléculaire de nouveaux composés ciblant les leucémies myéloïdes aigües de mauvais pronostic.

Authors: Camille Aitchedji, Dr. Jean-François Spinella, Deanne Gracias, Tara McRae, Dr Céline Moison, Dr. Simon Fortier Dr. Rodrigo Mendoza-Sanchez, Dr. Réjean Ruel, Dr. Josée Hébert, Dr Anne Marinier et Dr. Guy Sauvageau

Affiliation: Unité de recherche en génétique moléculaire des cellules souches, Institut de recherche en Cancérologie et Immunologie (IRIC), Département de Médecine, Université de Montréal, Montréal, Canada

Keywords: LMA ; découverte du médicament ; chémogénomique ; criblage génétique ; séquençage à haut débit

Background information: Malgré l'émergence de nouvelles thérapies ciblées, la leucémie myéloïde aigüe (LMA) reste un cancer de mauvais pronostic où seulement 20% des patients ont une espérance de survie supérieure à 5 ans. Dans le but de développer de nouvelles stratégies thérapeutiques, un criblage de 10000 composés a été effectué sur des échantillons primaires de patients atteints de LMA. Les composés testés ont été regroupés et sélectionnés en fonction de leur profil d'inhibition, réunissant les composés ayant une activité biologique similaire : les CCCs (Compound Correlation Clusters).

Purpose: L'objectif de ce projet est d'identifier la cible ainsi que le mécanisme d'action associé à l'un de ces CCCs, le CCC163, qui a une activité sélective sur les échantillons primaires de LMA. Parallèlement, nous recherchons un biomarqueur associé à la sensibilité et/ou la résistance aux composés du CCC163 dans l'optique de futures applications thérapeutiques en LMA.

Methods : Différentes stratégies ont été développées afin d'identifier la cible et le mécanisme associé à l'action du CCC163, incluant un criblage perte de fonction CRISPR/Cas9 sur le génome entier en présence des composés d'intérêt. Ces résultats ont été validés à

l'aide de shRNAs et/ou sgRNAS. En parallèle, un transcriptome réalisé sur une lignée cellulaire de LMA exposée ou non aux composés a été réalisé. Finalement, afin d'identifier un biomarqueur associé à la sensibilité ou à la résistance des échantillons de LMA, un composé du CCC163 a été testé en dose-réponse sur un panel de 124 échantillons primaires représentant la diversité génétique de la LMA.

Results : Le screen CRISPR/Cas9 a identifié le complexe Médiateur, complexe impliqué dans la régulation des l'expression des gènes comme la cible moléculaire. L'inactivation par shRNA et sgRNA des protéines de ce complexe a démontré la perte d'activité des composés du CCC163 dans des lignées LMA confirmant le complexe Médiateur comme la cible de ces composés. L'inhibition de CDK8, kinase régulatrice de l'activité transcriptionnelle du complexe Médiateur, est suggérée comme principal mécanisme d'action des composés. Des essais *in vitro* ont ainsi confirmés l'inhibition de l'activité catalytique de cette kinase par les molécules du CCC163. Des essais physico-chimiques ont également confirmé l'interaction de CDK8 avec les composés du CCC au sein des lignées LMA. D'autre part, l'analyse d'un transcriptome dans des cellules AML en présence des composés du CCC163 a révélé une diminution de l'expression de gènes connus pour être régulés par CDK8. Finalement, nous avons déterminé que la sensibilité au CCC163 est associée à des mutations sur FLT*3* alors que les mutations sur *TP53* sont associées à une résistance, dans nos 124 spécimens de LMA.

Conclusion : Ce projet a permis d'identifier le Médiateur complexe comme la cible moléculaire du CCC163 ainsi que le mécanisme d'action qui lui est associé. Cette découverte met en évidence une vulnérabilité de certains spécimens de LMA et identifie une nouvelle cible thérapeutique pertinente en LMA.

Title: Function and regulation of BAP1/ASXLs complexes and implications in the development of leukemia.

Author: Ali Boubacar Kalidou

Affiliation: Dr. El Bachir Affar, Department of Medicine, Maisonneuve Rosemont research center (HMR-CR) - University of Montreal.

Keywords: Leukemia, ASXLs, BAP1, Epigenetics, Tumor suppression.

Background information: ASXLs (Additional Sex Combs-Like proteins) proteins are among the most frequently mutated genes in myeloid malignancies and are associated with poor prognosis in childhood leukemia. They undergo truncations and inactivating mutations, which are observed in 10% of acute myeloid leukemia cases, 16% of myelodysplastic syndromes, and 45% of chronic myelomonocytic leukemia

cases. These epigenetic regulators interact with the tumor suppressor BAP1— a ubiquitously expressed deubiquitinase (DUB) that regulates chromatin-associated processes and is primarily involved in the deubiquitination of histone H2AK119. Inactivating mutations in BAP1 have also been found in multiple cancers, and this DUB is now established as a major tumor suppressor and the most frequently mutated DUB in mammalian malignancies, including chronic myelomonocytic leukemia. Mechanistically, BAP1 assembles DUB complexes (PR-DUB complex) with ASXLs in a mutually exclusive manner. Recently, our team has demonstrated that BAP1 interaction with ASXLs promotes their monoubiquitination. This post-translational modification regulates ASXLs stability, stimulates the DUB activity of BAP1, and is required for cell proliferation. However, new results from our laboratory suggest that another coordination mechanism of BAP1/ASXLs complexes is at work. These data show that BAP1 is monoubiquitinated on its catalytic domain on lysine 127 (K127) in a mutually exclusive manner with the monoubiquitination of ASXLs, possibly ensuring a fine-tuning of this epigenetic complex.

Purpose: 1) Investigate the roles of lysine 127 monoubiquitination of BAP1 in leukemia development in mice. **2)** Investigate, in vitro, the role of BAP1 monoubiquitination in the formation and function of the PR-DUB complex. **3)** Identify the ubiquitin ligase responsible for BAP1 monoubiquitination and characterize its cellular role.

Methods: We performed immunoprecipitation followed by mass spectrometry to identify the site of BAP1 monoubiquitination. Additionally, we used CRISPR/Cas9 to generate BAP1-K127R mutant mice. Finally, we will employ proximity-labelling assays (BirA), RNA-Seq, ChIP-Seq, and CUT&Run to, respectively, identify the ubiquitin ligases responsible for BAP1 monoubiquitination and assess the associated changes in the epigenetic landscape and gene expression.

Results: Our results show that BAP1 monoubiquitination regulates cell proliferation and could therefore coordinate the expression of genes involved in cell cycle progression. In addition, we generated “Knock-In” mutant mice with the BAP1-K127R mutation. Our observation of these animals indicates greater longevity in the mutated animals compared to wild-type animals, emphasizing the physiological importance of this mechanism of BAP1 monoubiquitination. Our data also suggest that BAP1-K127R mutant mice develop more cancers than WT mice, in particular liver cancer and leukemia.

Conclusion: We expect to determine an important role for BAP1 monoubiquitination in the suppression of leukemia, via proper regulation of cell proliferation. Overall, this project will undoubtedly further our

understanding of the regulation of BAP1-ASXLs complexes and their role in the protection against leukemia development.

Title: Co-design of a Combined Remote Symptom Monitoring and Self-management Support Program Post-Allogeneic Hematopoietic Stem Cell Transplantation: A Feasibility Study

Author: Sarah Chehayeb

Affiliation: Dr. Sylvie Lambert, Ingram School of Nursing, McGill University, Montreal, Canada

Keywords: Hematologic cancers, allogeneic hematopoietic stem cell transplantation, remote symptom monitoring, self-management support, feasibility.

Background information: Remote Symptom Monitoring (RSM) allows patients to electronically report symptoms (e.g., via a mobile app or email) using patient-reported outcome measures (PROMs) for real-time clinician feedback between clinic visits. Use of PROMs, like self-rating symptoms on a 0–10 scale, can improve patient-clinician communication, patients' health outcomes, and Quality of Life (QOL). However, patients with hematological cancers are often excluded from RSM programs due to the complexities of treatments like allogeneic hematopoietic stem cell transplantation (allo-HSCT). Despite the significant symptom burden faced by patients and caregivers and the need for daily self-management to prevent complications, no RSM program exists for post-allo-HSCT. Effective implementation requires tailoring PROM schedules and follow-up protocols while addressing challenges like time and resource constraints.

Purpose: 1) co-design the RSM program's content and implementation strategies with patients post-allo-HSCT, their caregivers, and clinicians; and **2)** implement and evaluate the feasibility, acceptability, and fidelity of RSM post-allo-HSCT and its implementation strategies.

Methods: This study employs an integrated convergent mixed methods approach. **Phase 1:** The RSM program will be co-designed using experience-based co-design. Clinic observations will inform workflows, followed by co-design workshops with 20 post-allo-HSCT patients, their caregivers, and 15 clinicians/managers across two Quebec centers to determine priorities for the program (e.g., self-management roles and barriers). Thematic analysis of the workshops will shape a summary video. A 2nd round of workshops will validate the program and refine implementation plans, leading to a joint co-workshop for finalization. **Phase 2:** The program's acceptability, feasibility, and fidelity will be assessed with 22 patients and caregivers using pre-set benchmarks

(e.g., satisfaction, attrition). Quantitative data will undergo descriptive analysis, qualitative data thematic analysis, and findings will be integrated for interpretation enhancement.

Results: We will develop a scalable, sustainable, and patient-centred RSM program tailored for patients, caregivers, and clinicians post-allo-HSCT. RSM can improve patients' QOL post-allo-HSCT by reducing symptom severity through better identification and management. Our innovative approach combines co-design with end-users and implementation science to proactively tackle barriers to use. Building on 7-year experience with Electronic Implementation of Patient- and Caregiver-reported Outcomes Across Cancer Centres in Quebec (E-IMPAQ, an evidence-based content, e.g., symptom management algorithms, self-management factsheets), we focus on the "what," "why," and "how" of RSM to ensure readiness for real-world application. This study lays a strong foundation for broader evaluation and scaling, with potential for national spread.

Conclusion: An RSM program empowers patients and caregivers to manage critical treatment challenges, enhancing quality of life, while equipping clinicians with tools for effective symptom monitoring.

Title: Genome editing with the DNA-PK inhibitor AZD7648 induces a low frequency of copy number variations in Hematopoietic Stem and Progenitors Cells

Authors: Fanny-Mei Cloarec-Ung, Dr. D Schieppati, Dr. G Sauvageau, Dr. HM Sheppard, Dr. PM Lansdorp, Dr. DJHF Knapp

Affiliation: David JHF Knapp, Département de pathologie et biologie cellulaire, Institut de Recherche en Immunologie et Cancérologie, Université de Montréal

Keywords: Genome Editing, Leukemia, CRISPR, Hematopoietic Stem Cells

Background information: Precise genome editing holds substantial promise for both accurate disease modelling and for potential curative treatments for monogenic disorders. There has been a particular interest in therapeutic editing in the hematopoietic system due to the ability to transplant cells to give life-long grafts, and the relatively large number of monogenic disorders that could be treated by gene repair/replacement. While precision edits have been the most suitable solution for these challenges, their low efficiency has, until recently, limited the development of therapies. However, we previously showed that precise editing is possible with >90% efficiencies in hematopoietic stem and progenitor cells (HSPCs) by combining optimized donor designs and the DNA-PK inhibitor AZD7648. Importantly, this editing did not

substantially affect the high self-renewing fraction of cells, though some toxicity was observed in late progenitors. While predicted off-target analyses showed no off-target editing, inhibition of DNA-PK could also result in large-scale structural variations (SVs), raising safety concerns for therapeutic applications.

Purpose: Here we sought to further validate the safety of our modified editing protocol to validate its use for study and clinical applications.

Methods/Results: We designed a multivariate strategy to detect SVs associated with AZD7648. To assess genome integrity, we used Strand-seq, a single-cell sequencing technique providing strand-specific signals for higher confidence when calling SVs (e.g., inversions, deletions, translocations). This method enabled chromosome-specific mapping for each HSPCs sequenced. Single and dual editing at SRSF2 and DNMT3A loci were compared using classic and AZD7648-enhanced protocols against non-edited controls. A Bayesian mixed effect model has been chosen to quantify structural variation rates across conditions, incorporating covariates like editing protocol, editing type, and sex. The Bayesian framework enabled robust estimation of event frequencies and credible intervals, providing a nuanced view of the data. Non-edited controls exhibited no detectable SVs (0 events/163 cells). Classic editing protocol showed rare SVs (8 events/263 cells), while AZD7648-enhanced editing, maintained low SV rates (16 events/328 cells), independent of single/dual editing. Interestingly, observed SVs were neither at target chromosomes nor consistent sites, suggesting no consistent off-target effects. A Bayesian analysis revealed no significant SV rate increase attributable to AZD7648 for DNMT3A but identified a slight increase for SRSF2. No significant sex effects were observed. All together, these data further support the protocol's safety and generalizability. In addition, long-read nanopore sequencing and qPCR around on-target sites were used to identify larger on-target deletions. A preliminary analysis of the nanopore data for the SRSF2 loci showed a slight increase in deletion events at the on-target site.

Conclusion: These results demonstrate that AZD7648-enhanced editing largely maintains genome integrity, with rare, target-dependent SVs across the genome and rare deletion events at on-target sites. Thus, careful target selection and screening are crucial for therapeutic applications to minimize genotoxicity.

Title: Characterization of TMT1B as a biomarker in KMT2A-MLLT3 acute myeloid leukemia

Author: Sarah Denford

Affiliation: Dr. Brian Wilhelm, Institute for Research in Immunology and Cancer (IRIC), Université de Montréal

Keywords: Protein characterization, BioID, Biomarker, Methyltransferase, KMT2A

Background information: Following the development of acute myeloid leukemia driven by the KMT2A-MLLT3 fusion in murine models and patient samples, several genes were found to be biasedly expressed in these cells compared to healthy or other leukemic cells. The effects of the upregulation of these genes following the introduction of the fusion were thought to have a role in the development or maintenance of the leukemia. Many biomarkers identified in the patient and model samples were found to be not fully characterized and their activities within the cell unknown.

Purpose: Thiol methyltransferase 1B (TMT1B, previously known as METTL7B), is a protein found to be biasedly expressed in our KMT2A-MLLT3 leukemic models, patient samples, and cell lines, as well as in several solid cancers. In many of these cases, overexpression of TMT1B is linked to poorer patient outcomes, increased proliferation and tissue invasion. We aim to characterize the mechanisms of action and functional effects of TMT1B in KMT2A-MLLT3 AML cells.

Methods: Using human acute myeloid leukemia cells expressing the KMT2A-MLLT3 fusion, an inducible-shutoff system for *TMT1B* was generated where the endogenous *TMT1B* and its closest gene relative, *TMT1A*, were both knocked out. With these cells, gene expression was analyzed to identify differentially expressed genes dependent on the expression of *TMT1B*. Additionally, microscopy was completed to validate the localization of TMT1B in KMT2A-MLLT3 AML cells to identify potential functions within the cell. Protein interactors of TMT1B were identified by protein proximity-dependent biotin labeling (BioID) in four KMT2A-MLLT3 AML cell lines (completed in technical triplicate) and one control non-KMT2Ar AML cell line, with those consistently interacting with the bait protein recognized and their activities compared to known effects of TMT1B expression.

Results: Transcriptomic analyses in the KMT2A-MLLT3 *TMT1B*-shutoff cells identified downregulated genes pertaining to cell division, including *CCNA1*, *CCNA2*, *CDC20* and *CDC25A*. Microscopy images of TMT1B's localization experiments within KMT2A-MLLT3 AML cells supports its activity in the cytoplasm, specifically in the endoplasmic

reticulum. From the interactome analysis, proteins involved in mRNA and protein processing as well as in cytoskeletal functions (cellular structure and migration) were identified to be consistent interactors of TMT1B.

Conclusion: In conclusion, we propose that TMT1B's activity as a methyltransferase induces several effects in KMT2A-MLLT3 AML cells that may promote or support leukemogenesis. The fact that TMT1B has been found to influence such a diverse group of pathways could indicate vulnerabilities that can be targeted with pharmacological inhibitors. No direct inhibitors for TMT1B have previously been described, however with TMT1B's rising interest as a putative oncogene and biomarker in both liquid and solid cancers, this would represent a novel potential therapeutic target.

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

Author: Roudy Farah

Affiliation: Dr. Moutih Rafei, Department of Microbiology, Infectious Diseases and Immunology, Université de Montréal

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

Background information: Unlike normal cells, leukemic cells undergo DNA modifications leading to the expression of cancer antigens. In fact, multiple screening studies corroborate their elevated expression levels in leukemia 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 leukemia-associated antigens.

Purpose: 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 will be 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 will be assessed against various cancer cell

types (EL4, E0771, X577, Pan02, and B16) in immunocompetent C57BL/6 mice.

Results: The safety and tolerability of 8 *ex vivo* pharmacological treatments have already been confirmed. The MTD of each drug was determined following 48h and 72h treatments and 2 doses were selected per drug for annexin V staining to determine the concentration inducing the least apoptosis. MHC class I quantification following treatments revealed increased levels in a dose-dependent manner. Furthermore, combination treatments are currently being tested for tolerability.

Conclusion: This research could enhance our understanding of certain cancer antigens as well as 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

Author: Dr. Laurence M. Gagné

Affiliation: Dr. Frédérick Antoine Mallette, Division of Immunology-Oncology, Maisonneuve-Rosemont Hospital Research Center, Department of Biochemistry and Molecular Medicine, Université de Montréal

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, α-ketoglutarate (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 showed that KDM4A localizes at telomeres and that its depletion or inhibition by *R*-2HG leads to telomere defects, suggesting that KDM4A plays a role in telomere stability.

Purpose: We hypothesize that *R*-2HG leads to telomere dysfunction by impairing DNA replication at telomeres via KDM4A catalytic domain

inhibition inducing genomic instability in blast cells that contribute to AML development.

Methods: To evaluate the impact of IDH1/2 mutation, which produces the oncometabolite *R*-2HG, on telomeres, we used cells transduced with $IDH1^{WT}$ or $IDH1^{Mutated}$ or treated with *R*-2HG. Using these cells, we assessed telomere integrity by performing telomere FISH on metaphase spreads and evaluated DNA replication through a DNA fibre assay and EdU incorporation, followed by FISH/IF staining. We conducted similar experiments with cells depleted of KDM4A or treated with the KDM4 inhibitors QC6352 and ML324. To monitor KDM4A association with telomeres, we performed ChIP-seq experiments, immunoprecipitation, and GST pull-down assays.

Results: We demonstrate that exposure to *R*-2HG or KDM4A depletion induces senescence, relying on the presence of the p53 protein. KDM4A, in addition to localize at telomeres, regulates levels of telomeric H3K9(me3). KDM4A is also interacting with the shelterin complex, especially with TRF2 through its PHD domain. Interestingly, rescue experiments with the catalytically dead mutant or 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. Additionally, *R*-2HG exposed or KDM4A-depleted cells show reduced progression of replication forks.

Conclusion: This study elucidates the role of methylation in telomeres regulation and improve our knowledge of the early events leading to gliomas related to the effect of genomic instability on telomeres. Glial tumors are often refractory to conventional treatments, making it necessary to discover new therapeutic avenues.

Title: Characterization of the role of eIF4A in the resistance of acute myeloid leukemia cells to chemotherapy and targeted therapies

Authors: Victor Gife[1,2], Elodie Petrecca[3], Dr. Bahram Sharif-Askari[4], Anavasadat Sadr Hashemi Nejad[1,2], Dr. Raquel Aloyz[3,4], Dr. Laura Hulea[1,2*] and Dr. François Mercier[3,4*]

Affiliation: [1]Centre de recherche de l'hopital Maisonneuve-Rosemont, Montréal; [2]Département de Biochimie et Médecine Moléculaire, Université de Montréal; [3]Faculty of Medicine McGill University, Departments of Experimental Medicine, Medecine and Oncology, Montréal; [4]Lady Davis Institute for Medical Research, Montreal; *equal contribution

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

Background information: Acute myeloid leukemia (AML) is a cancer of hematopoietic progenitor cells in the myeloid lineage, characterized by uncontrolled proliferation and impaired differentiation. Despite advances in chemotherapy, targeted therapies, and stem cell transplantation, the 5-year survival rate remains around 30%, with poorer outcomes in older patients or those with high-risk genetic profiles. A major challenge in AML treatment is the emergence of drug resistance, leading to relapse and therapy failure. This resistance has been linked to metabolic reprogramming, driven by transcriptional and post-transcriptional changes that enhance metabolic plasticity (oxidative phosphorylation, glycolysis). Aberrant mRNA translation is a key feature of many cancers, including AML. The eIF4F complex, composed of eIF4A, eIF4E, and eIF4G, is regulated by mTORC1, which controls translation initiation. Among these factors, eIF4A, the helicase of the complex, has emerged as a promising therapeutic target to counteract treatment resistance in AML.

Purpose: Previous studies have shown reactivation of the mTORC1 pathway following cytarabine treatment in an in vivo AML mouse model. We propose that leukemia cells exposed to treatment activate several translationally controlled metabolic pathways through eIF4A to adapt and survive.

Methods: Using specific inhibitors of eIF4A (Zotatifin, CR-1-31-B) in established MOLM14 cytarabine-resistant AML cell line, we have shown affecting viability, bioenergetic metabolism and intracellular metabolite balance. Furthermore, in PDX model we show a strong synergetic effect combined with venetoclax, a specific inhibitor of the anti-apoptotic protein BCL2, already used in the treatment of AML and in cases of relapse. Where with a new in vivo protocol developed, analysing mitochondria statement, anti-apoptotic proteins expression and activation of intracellular key pathways, we can present the importance of all these underlying molecular mechanism in the resistance becoming face of treatments.

Results: Our new results help in the understanding of molecular underlying mechanisms used by the AML cells to adapt and resist to treatments.

Conclusion: Inhibiting specific translation by eIF4Ai seems to be a good new approach, combined with other approved treatment, to reduce relapsed cases in patients.

Title: Multi-Functional T Cells for Precision Immunotherapy

Authors: Dr. Leila Jafarzadeh, Dr. Sabrina Guettouche, Béatrice Portier, Ali Smaani, Gabrielle Boudreau, Dr. Cédric Carli, Dr. Jean-Sebastien Delisle

Affiliation: Jean-Sebastien Delisle lab, Devision of Immunology-Oncology, Maisonneuve-Rosemont Hospital Research Centere; University of Montreal.

Keywords: CAR T, Tacrolimus, FKBP12, Blood Cancers, CRISPR-Cas9

Background information: Allogeneic Hematopoietic Cell Transplantation (AHCT) is a critical treatment for hematologic malignancies, yet its efficacy is hindered by disease relapse, graft-versus-host disease (GVHD), and prolonged immunosuppression. Tacrolimus, a standard immunosuppressant, broadly inhibits T-cell function. Disrupting FKBP12, the intracellular binding partner of Tacrolimus, offers a strategy to generate Tacrolimus-resistant T cells while preserving their function.

Purpose: This study aims to engineer multifunctional T cells resistant to Tacrolimus by knocking out FKBP12 while incorporating virus-specific and anti-CD19 CAR functionalities, enhancing post-AHCT immune reconstitution.

Methods: Peripheral blood mononuclear cells (PBMCs) from healthy donors were genetically modified via CRISPR-Cas9 to delete FKBP12, followed by lentiviral transduction with an anti-CD19 CAR construct. Cells were stimulated with anti-CD3/CD28 antibodies or viral peptide-loaded dendritic cells and expanded in the presence or absence of Tacrolimus (10 ng/ml). Functional assays assessed cytokine expression (IFN-γ, TNFα, IL-2), cytotoxicity, and T-cell phenotype (CD45RA, CCR7, PD-1, TIM-3, 2B4, TIGIT, LAG3). The multifunctionality of FKBP12KO CAR T cells was evaluated after repeated stimulations (days 15, 18, 21, and 24) using NALM6 target cells (CD19 expressing cells) and peptide-loaded PBMCs to assess sustained responsiveness.

Results: FKBP12-knockout (FKBP12KO) T cells maintained robust expansion and cytokine production despite Tacrolimus exposure, while non-edited cells exhibited impaired function. Engineered FKBP12KO CAR T cells demonstrated effective cytotoxicity against CD19-expressing targets and retained virus reactivity. Multiple restimulations confirmed sustained functionality, as FKBP12KO CAR T cells continued to produce cytokines (IL-2, IFN-ɣ, TNF-α) and displayed stable dual specificity to both viral peptides (TCR activation) and CD19 (CAR activation). Multifunctional T cells persisted and remained functionally competent, with no evidence of progressive dysfunction upon repeated stimulation. Differentiation analysis showed that the majority of cells

differentiated into effector memory T cells (Tem), with FKBP12 knockout having no significant impact on differentiation patterns.

Conclusion: FKBP12 knockout successfully generates Tacrolimus-resistant T cells with preserved antiviral and anti-tumor functionality, offering a promising approach for post-AHCT immunotherapy. Future studies will explore in vivo efficacy and clinical translation.

Title: Treatment of Hematological Malignancies via TCR-T cells engineering to target Minor Histocompatibility antigens

Authors: Nafiseh Keshavarzian, Annabelle Minguy, Dr. Nasser Masroori, Dr. Jean-Philippe Bastien, Jessica Trottier, Jaime Sanchez-Dardon, Dr. Vibhuti Dave, Dr. Denis-Claude Roy, department of Medicine, CRHRM

Affiliation: Hopital Maisonneuve-Rosemont Research Centre, and Department of Medicine Division of Molecular Biology, and Department of Medicine, Université de Montréal, Montreal, Québec, Canada

Background information: Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative option for most patients with high-risk or relapsing hematologic malignancies. Donor T cells within the graft may mediate the beneficial graft-versus-leukemia (GvL) effect by recognizing antigens, such as minor histocompatibility antigens (MiHAs), presented by malignant cells. Cellular therapies aiming to enhance GvL effect are currently being developed. This includes the ex vivo genetic modification of T cells to improve their ability to recognize MiHAs expressed on hematopoietic cells. However, the strength of the GvL effect can vary depending on the targeted antigen and the donor source.

Purpose: To optimize MiHA targeted therapy previously isolated and sequenced MiHA-specific TCRs for three different antigens presented on HLA-A02 and HLA-B07 molecules were tested on different donors.

Methods: These TCRs were then transduced CD8+ T cells from fourteen healthy donors. The engineered T cells were re-stimulated with antigen-presenting cells (APCs) pulsed with their cognate MiHA. T cell functionality was then assessed using several measures such as production of IFN-ɣ and TNF-α, expression of degranulation marker CD107a, and ability to eliminate MiHA-expressing autologous blast cells or target cells.

Results: In this study, we show that engineered T cells effectively responded to both MiHA presenting DCs and malignant target cells with DC exerting stronger immune responses. Interestingly, T cells from one donor consistently exhibited greater cytokine secretion, CD107a

expression, and cytotoxicity compared to T cells from other donors. This difference was observed in all tested TCRs. This indicates that donor-intrinsic factors may influence the MiHA-specific response. Additionally, the choice of targeted antigen also impacted the anti-MiHA activity as T cells from the same displayed differential functionality were observed when challenged with different MiHA. This suggests that variations in TCR affinity for the cognate MiHA may play a significant role in response outcomes.

Conclusion: In conclusion, these findings demonstrate that the efficacy of engineered T cells is influenced by multiple factors, including the donor source and the specific interactions between TCRs and MiHAs. A deeper understanding of these variables will aid in selecting optimal donors and TCRs, ultimately enabling the development of highly potent engineered MiHA-specific TCR-T cells for the treatment of hematologic cancers.

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

Author: Tyler Lussier

Affiliation: Dr. Brian Wilhelm, Institute for Research in Immunology and Cancer (IRIC), Université de Montréal

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

Background information: Acute myeloid leukemia (AML) represents ~20% of pediatric leukemia cases. The prognosis of this disease remains poor, with a ~60% 5-year survival rate, largely due to disease relapse initiated from a subpopulation of chemo-resistant leukemic cells. This subpopulation often consists of quiescent cells, which are dormant cells that have exited the cell cycle. The bone marrow microenvironment, or niche, has become increasingly appreciated as an important regulator of drug resistance and quiescence. However, the precise mechanisms, especially those regulating quiescence, are underexplored.

Purpose: Our aim is to identify interactions between AML cells and their microenvironment that leads to resistance towards common chemotherapeutics and to identify the scope of this resistance by testing other anti-AML compounds.

Methods: To model the AML microenvironment, we use a co-culture model between AML cell lines and HS-5, a human bone marrow stromal cell line, in both direct and indirect co-culture with a 0.4µm pore-size transwell (TW) insert. We have carefully adapted the HS-5 cells from DMEM to RPMI 1640 and IMDM growth medias, which are

recommended for many AML cell lines. To characterise phenotypic changes in co-culture, we used flow cytometry. This allows us to specifically measure the AML cells either by using cell lines with fluorescent protein reporters or with an antibody against a protein expressed by the AML cells and not HS-5, CD33 or CD45. We have used these strategies to measure the proliferation of both cell types as well as the cell cycle of AML cell lines by PI-Ki67 co-staining and apoptosis after cytarabine treatment by annexin V-PI co-staining.

Results: Firstly, we have replicated in two AML cell lines (THP-1 and SHI-1) the previously described HS-5-mediated resistance to cytarabine. Additionally, we observed that AML cell lines (THP-1 and NOMO-1) as well as HS-5 cells proliferate slower in co-culture compared to their monoculture. Furthermore, in our cell cycle analysis, we observed an increase of the proportion of THP-1 cells in the G0/G1 phase in both direct and TW co-culture. This increase in G0/G1 corresponded to an increase in quiescent Ki67 negative G0 cells. However, this effect is not as strong for SHI-1/HS-5 co-cultures and is only significant with TW inserts. This optimized model will allow us to adapt to a high-throughput format to re-screen 70 anti-leukemic compounds previously identified in our lab to determine the scale of the drug resistance effect. We will also be able to further probe interactions that lead to drug resistance by performing a CRISPR chemogenomic screen of 229 computationally selected AML surface proteins.

Conclusion: In conclusion, we have established an optimized co-culture system and have begun to characterize the phenotypic alterations that arise in the AML cells. Most notably, we have observed an increase in quiescence in at least one cell line, which is potentially contributing to the observed drug resistance, but this will be studied further. Ultimately, this work will help identify proteins and mechanisms regulating the observed AML chemo-resistance in HS-5 co-culture and could potentially identify regulatory mechanisms for AML quiescence.

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

Author: Pault Y Minaya Ferruzo

Affiliation: Dr. Frédérick A. Mallette. Centre de recherche de l'Hôpital Maisonneuve-Rosemont (CRHMR). Dépt. Biochimie, Université de Montréal.

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. Resistance to standard treatments underscores the need for novel therapies to halt progression and relapse. 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, 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: We propose that dual targeting of KDM4A and eIF4A in pediatric AML will eradicate malignant cells and limit residual disease persistence.

Methods: We investigated whether a sequential regimen, starting with QC6352 (KDM4A inhibitor) followed by CR-1-31B (eIF4A inhibitor), could enhance survival and extend disease-free intervals in AML models. In vitro, we used AML cell lines (THP1, MonoMac1, MOLM14). In vivo, we will assess efficacy in two models: immunodeficient NSG mice engrafted with human AML xenografts (THP1 or MOLM14) will receive sequential or combined therapy with survival monitored, and a syngeneic AML model (RN2 in C57BL/6J mice) will determine the effect of a functional immune system on treatment outcomes.

Results: In vitro, THP1 and MOLM14 cells demonstrated increased sensitivity to sequential treatment with QC6352 and CR-1-31-B. 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.

Title: Le traitement personnalisé de la leucémie lymphoblastique aiguë chez les enfants ayant une toxicité induite par l'asparaginase

Author: Covida Mootoosamy

Affiliation: Dr. Maja Krajinovic, CRCHU Sainte-Justine, Université de Montréal)

Keywords: Pharmacogénomique, Asparaginase, Toxicité, Pancréatite Aïgue, Criblage Pharmacologique

Background information: La leucémie lymphoblastique aiguë (LLA) est le cancer pédiatrique le plus fréquent, avec des taux de survie dépassant 90% grâce à l'optimisation des traitements. Cependant, les réactions indésirables graves aux médicaments demeurent une source majeure de morbidité, affectant environ 50% des patients traités. L'asparaginase, un médicament clé dans le traitement de la LLA, est associée à des réactions indésirables importantes, telles que la pancréatite, la thrombose et l'allergie, limitant son utilisation optimale. Les variants génétiques jouent un rôle majeur dans la distinction entre les patients sensibles et non sensibles au développement des réactions indésirables. Nos études pharmacogénomiques ont identifié des variants germinaux associés aux toxicités liées à l'asparaginase. Parmi ces variants, le polymorphisme nucléotidique rs11556218 dans le gène *IL16* a été associé à des complications graves liées à l'asparaginase, notamment la pancréatite et la thrombose.

Purpose: L'objectif de cette étude est d'identifier un médicament capable de réduire la sensibilité associée au polymorphisme rs11556218, et ainsi de diminuer la toxicité induite par l'asparaginase chez les patients.

Methods: Pour ce faire, nous avons mené un criblage pharmacologique en utilisant des lignées cellulaires lymphoblastoïdes (LCLs) portant l'allèle de risque de rs11556218. Notre objectif était d'identifier des composés capables de restaurer la viabilité cellulaire en présence d'asparaginase.

Results: Ce criblage, réalisé à l'aide de la bibliothèque Pharmakon contenant 1 760 médicaments approuvés par la FDA ou l'EMA, a permis d'identifier plusieurs composés prometteurs. Parmi eux, la glycyrrhizine s'est révélée particulièrement efficace. La glycyrrhizine, un dérivé naturel de la réglisse, possède des propriétés anti-inflammatoires, antioxydantes et protectrice contre la pancréatite aïgue. Nos résultats montrent que la glycyrrhizine augmente significativement la viabilité des cellules de quatre LCLs portant rs11556218 en présence d'asparaginase, suggérant son potentiel pour atténuer les effets indésirables

chez les patients pédiatriques atteints de LLA. Par la suite, nous avons évalué l'impact de la glycyrrhizine sur des marqueurs pro-inflammatoires, en effectuant un test ELISA sur une LCL portant l'allèle de risque de rs11556218. Ce test a permis de mesurer les niveaux de plusieurs cytokines pro-inflammatoires afin de déterminer si l'ajout de glycyrrhizine à l'asparaginase réduisait ces biomarqueurs par rapport à l'asparaginase seule. Nos résultats montrent que la glycyrrhizine diminue significativement les niveaux de cytokines pro-inflammatoire IL6, IL8, TNFα, IL5 et IL12p40 des cellules de LCL portant rs11556218 en présence d'asparaginase.

Conclusion: Ces données ouvrent des perspectives prometteuses pour l'intégration de la glycyrrhizine dans les protocoles de chimiothérapie afin d'améliorer la tolérance au traitement et les résultats cliniques.

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

Author: Marcelo Muniz Corrêa

Affiliation: Dr. John Pascal Laboratory, Département de Biochimie et Médecine Moléculaire, Université de Montréal

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

Background: Poly(ADP-ribose) polymerase 1 (PARP1) is the major contributor to PAR synthesis in cells and plays a key role in the cellular response to DNA damage. Binding to DNA breaks triggers PARylation of proteins by PARP1, which in turn recruits DNA repair factors. Cancers carrying specific deficiencies in DNA repair genes are dependent on PARP1-mediated repair pathways, which can be 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 combination with cytotoxic chemotherapy 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, unveiling 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 and surface plasmon resonance DNA binding assays, and hydrogen/deuterium exchange mass spectrometry to characterize the allosteric effects of novel PARPi on PARP1 bound to damaged DNA. We will gain atomic-level insights on DNA-bound, full-length PARP1 in the presence of PARPi by cryo-electron microscopy (cryo-EM). In parallel studies, we will assess the activity of new PARPi in two leukemia cell lines: KASUMI-1 and THP-1. KASUMI-1 harbours the AML1-ETO fusion oncogene, which leads to several malfunctioning DNA repair proteins, resulting in PARPi monotherapy sensitivity. THP-1 cells bear the MLL-AF9 translocation thus affording them with limited sensitivity to PARPi, which can be overcome if combined with other cytotoxic agents. CD34+ bone marrow cells will serve as controls to assess potential cytotoxicity of novel PARPi. PARP1 retention on DNA at the cellular level will be measured using chromatin fractionation assays as a function of PARPi.

Preliminary/Anticipated Results: Our recent cryo-EM studies unveiled previously unseen details of full-length PARP1 structure bound to binding partners HPF1 and Timeless. To overcome inherent challenges during cryo-EM sample preparation, namely sample degradation and preferential orientation of our protein complexes, we are employing the air-water interface partitioning protein LEA (Late Embryogenesis Abundant) as well as shorter damaged DNA probes. We anticipate that these strategies will also aid in obtaining improved resolution for the mobile regions of PARP1's DNA binding domains.

Conclusion: 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: Genetics of 6-MP related toxicities in pediatric patients with acute lymphoblastic leukemia

Author: Zarina Sabirova

Affiliation: Dr. Maja Krajinovic. Department of Pharmacology and Physiology, Faculty of Medicine, University of Montreal and CHU Ste – Justine Research Center.

Keywords: acute lymphoblastic leukemia, pharmacogenomics, 6-mercaptopurine, genetic variants, adverse events.

Background information: Acute lymphoblastic leukemia (ALL) is the most common cancer in children. Over the past six decades, the refinement of dosing, scheduling, and combinations of chemotherapy

agents significantly improved the prognosis of childhood ALL. However, treatment related adverse drug reactions (ADRs) remain a significant threat to the well-being of many children. Severe myelosuppression, commonly associated with 6-mercaptopurine (6-MP) can lead to life-threatening infections or frequent treatment interruptions, potentially compromising treatment efficacy. High levels of the 6-thioguanine (6-TGN) metabolite worsen neutropenia, while the accumulation of the 6-methylmercaptopurine (6-MMP) metabolite is linked to hepatotoxicity. Genetic variations can influence a child's susceptibility to ADRs by modulating the effects of drugs used in ALL treatment.

Purpose: Identify and analyze additional variants within the 6-MP pathway, apart from the already recognized variants in *TPMT* and *NUDT15* genes, in the context of 6-MP toxicity among pediatric ALL patients.

Methods: This study examines the tag SNPs within the *GMPS*, *IMPDH*, *XO*, and *ITPA* genes, along with a newly identified variant in the *NUDT15* gene. These genes encode enzymes crucial in the 6-MP pathway. Genotypes were derived from germline whole-exome sequencing (WES) data from the SIGNATURE project or through allele-specific PCR. These genotypes were then correlated with various clinical and pharmacologic outcome measures in ~300 ALL patients treated at CHU Sainte Justine (CHUSJ) with Dana Farber Cancer Institute (DFCI) protocols 05-00, 11-01, and 16-001. Pharmacologic outcomes include 6-MP dose intensity, expressed as the ratio between received and predicted drug dose and levels of 6-MP metabolites, 6-TGN and 6-MMP. Clinical parameters include hepatotoxicity and hematological toxicity, such as high-grade neutropenia.

Results: Our study findings indicate that genetic variations within the *XO* and *IMPDH* genes as well as the newly identified variant in the *NUDT15* gene have an impact on the response to 6-MP treatment. The results reveal that carriers of at least one copy of the rs1884725 variant allele in the *XO* gene is associated with a reduction of neutropenia and higher total received doses of 6-MP. The rs6710015 variant allele in the same gene has been associated with lower 6-TGN levels. In contrast, an opposite and anticipated effect was found for the 6-MMP metabolite. Meanwhile, two variants in the *IMPDH* gene, rs2228075 and rs2278294, have been correlated with a higher frequency of neutropenia during the consolidation II and maintenance phases. Additionally, a novel variant, rs73189762, in the *NUDT15* gene has been associated with increased neutropenia frequency.

Conclusion: As advancements in the treatment of ALL has led to higher success rates, the research focus is shifting towards improving the short-term and long-term quality of life for patients. A crucial aspect

of achieving this goal involves reducing the occurrence of side effects related to the therapy. Personalized treatment strategies, such as identifying genetic factors influencing treatment responses in ALL are essential components in achieving this goal.

Title: Exploring The Potential Of CAR-Engineered HSCs For Treating B-cell Acute Lymphoblastic Leukemia

Author: Clara Soulard

Affiliation: Dr. Élie Haddad, CHU Sainte-Justine Research Center, Université de Montréal

Keywords: B cell acute lymphoblastic leukemia, Hematopoietic stem cells, Cell-type specific promoters, CAR, Humanized mice

Background information: The adoptive transfer of T and NK cells modified to express chimeric antigen receptors (CARs) has emerged as a promising therapy for the treatment of B-cell acute lymphoblastic leukemia (B-ALL). However, challenges such as poor proliferation, loss of engineered cells, and cell exhaustion limit its efficacy.

Purpose: We hypothesized that transducing CAR in hematopoietic stem cells (HSCs), instead of mature immune cells, could provide a continuous supply of CAR-modified cells.

Methods and Results: NSG (NOD/SCID/IL2Rɣ null) mice were humanized with HSCs transduced with a CAR targeting CD22 under the control of the human elongation factor-1 (EF1)-α promoter. We demonstrated that CAR expression in T-cell progenitors disrupted T-cell development, resulting in the absence of peripheral CAR-T cells. Additionally, constitutive CAR expression across the hematopoietic compartment increased the risk of proto-oncogene activation. To overcome these challenges, we developed a synthetic T/NK-specific promoter to restrict CAR expression to mature T and NK cells. Using a Green Fluorescent Protein (GFP) reporter gene under the synthetic promoter's control, we validated its specificity in mature T and NK cells after *in vitro* and *in vivo* HSC differentiation. Next, we humanized mice with HSCs transduced with a CAR-CD22 driven by the T/NK-specific promoter. Substituting the EF1-α promoter with the T/NK promoter overcame the CAR-T differentiation blockade, enabling the generation of HSCs derived mature CAR-T cells. Moreover, when challenged with B-ALL cells, mice engrafted with CAR-engineered HSCs under the T/NK promoter exhibited delayed leukemia progression and significantly improved survival. Interestingly, mice engrafted with HSCs under the EF1-α promoter also showed improved survival, despite the absence of CAR-T cells, suggesting a role for CAR-NK and/or CAR-macrophages in leukemia control. This raises the question of which cell types should be targeted for CAR expression in a context of CAR HSCs, when persistence of CAR-expressing cells is

no longer an issue. To investigate, we have developed a high-throughput method to screen a large number of lineage-specific promoters. A lentiviral library of fifty promoters controlling GFP expression was used to transduce T, B, NK cells, and macrophages isolated from human peripheral blood mononuclear cells (PBMCs). GFP+ populations, containing cells with active promoters, were sorted *via* fluorescence-activated cell sorting. Long-read nanopore sequencing were used to determine the enrichment of each promoter sequence in the GFP$^+$ T, B, NK cells, and macrophages. The sequencing results showed a strong correlation with those obtained from individual promoter transduction experiments in human PBMCs, confirming the robustness of the approach.

Conclusion: The improved survival observed with the T/NK-specific promoter highlights the potential of CAR-HSC therapy. Expanding the repertoire of available cell-specific promoters will allow further evaluation of each cell type's contribution to leukemia control, paving the way for more efficient CAR-HSC strategies.

Title: Characterization of the proteomic signature in response to apoptosis inducers in pediatric acute megakaryoblastic leukemia

Author: Nadia Emely Chauca Torres

Affiliation: Dr. Sonia Cellot, Centre de recherche Azrieli du CHU Sainte-Justine, Université de Montréal

Keywords: leukemia; apoptosis; proteome; resistance; cell death

Background information: Resistance to regulated cell death is a hallmark of cancer, promoting malignant cell survival and limiting treatment efficacy. Proteins that block or inhibit apoptosis contribute to this resistance by blocking caspase activation and modulating death receptor signaling. High-risk pediatric acute megakaryoblastic leukemia (pAMKL) subtypes, such as *CBFA2T3::GLIS2* (CG2) and *NUP98::KDM5A* (N5A), show poor prognosis and dysregulated cell death pathways. Our group have developed CG2 and N5A patient derived xenograft (PDX) and synthetic models to identify therapeutic vulnerabilities (Cardin *et al*., 2019; Gress *et al*., 2024). Through a high-throughput drug screening we have identified an apoptosis-inducing compound associated with cell- and genotype-specific therapeutic response (Safa-Tahar-Henni S., *Leukemia*, 2025).

Purpose: To investigate the molecular basis of differential drug response in pAMKL, we profiled proteomic changes in sensitive and resistant models. Our aim is to identify the mechanism of action and identify actionable pathways to guide rational drug combinations and overcome resistance.

Methods: Commercial cell lines harboring CG2 (M07e) and N5A (CHRF-288-11) gene fusions were treated with 0.5 µM and 1 µM of our candidate drug for 6 and 24 hours. Following treatment, approximately 2–3 × 10^6 cells per condition were collected, washed, lysed, and digested with trypsin. Peptide samples (1 µg) were analyzed by mass spectrometry using a data-independent acquisition (DIA) strategy at the proteomics platform. Identified peptides were matched against the canonical human proteome (UniProt database) using PEAKS software. Prior to differential expression and pathway enrichment analysis, data were filtered and normalized. Bioinformatic analysis was conducted in RStudio, with differentially expressed proteins (DEPs) defined by a threshold of $|\log_2 FC| > 0.5$ and p-value < 0.05.

Results: After 6 hours of treatment, M07e cells exhibited sensitivity to the drug, as expected. This was reflected in the downregulation of proteins involved in TNF–TNFR-dependent apoptotic signaling and NF-́B pathway regulation. Although TGF-́ signaling pathways were upregulated—suggesting a compensatory survival mechanism—this response was insufficient to overcome the drug's effect. In contrast, CHRF-288-11 cells demonstrated resistance to the treatment even after 24h exposure with an upregulation of the TNF–TNFR signaling and a downregulation of TGF-́ pathway, indicating the activation of alternative mechanisms associated with a markedly different response.

Conclusion: These preliminary findings highlight distinct molecular responses between sensitive and resistant models and pinpoint potential pathways to explore through combination drug strategies to sensitize leukemic cells and overcome resistance to cell death.

Title: Characterization of Mast Progenitors in Primary Acute Myeloid Leukemia

Author: Dr. Rui Zhang

Affiliation: Dr. Vincent-Philippe Lavallee, CHU Sainte-Justine Research Center/University of Montreal

Keywords: scRNA-seq, acute myeloid leukemia, mast progenitor, heterogeneity, aberrant differentiation

Background information: Acute Myeloid Leukemia (AML) is a highly heterogeneous disease characterized by a diverse mix of cell types and genetic alterations that is stratified into favorable, intermediate, and adverse group. Single-cell genomic techniques allow for a detailed analysis of an inter- and intra-patient heterogeneity by examining the gene expression profiles of individual cells, revealing distinct cell populations. Our group have profiled, using scRNA-seq, 192 primary AML samples from every major AML genetic subgroup. We annotated each

leukemic cell to its most similar normal cell type using a machine learning classifier we developed. We identified an expansion of mast progenitors within a subset of AML patients. In this study, we applied different computational tools on our scRNAseq AML cohort to characterize these rare cell population and explore their role in leukemogenesis.

Purpose: to expand our understanding of mast progenitors in AML.

Methods: To identify gene signatures that are specific for mast progenitors in AML samples, I applied unsupervised clustering analysis using Seurat package in R to the scRNAseq data of ten AML samples from t (8; 21), inv (16), normal karyotype, tMLL and EVI1-r subgroups. I further filtered all the marker genes and obtained the mast progenitor surface proteins by using the Surface Protein Annotation Tool. To determine mast progenitors' cellular origin in AML, we sorted mast progenitors from seven AML samples by flow cytometry for deep targeted sequencing. To further explore the heterogeneity of mast progenitors in AML, I subsetted all the mast progenitors from our scRNAseq AML cohort for differential gene expression (DEG) analysis.

Results: We identified FCER1A, MS4A2 and IL1RL1 as mast progenitor surface markers across most of the AML samples and were able to isolate mast progenitors by FACS using the antibodies against these cell surface markers. By doing deep targeted sequencing, we confirmed the sorted mast progenitors share very similar mutational profile with leukemic blasts (similar VAF in different mutated genes). By doing pseudobulk analysis for DEG, I identified 84 up-regulated and 246 down-regulated genes in mast progenitors from CBF-AML subgroup compared to those from other subgroups. Using a reference atlas of human bone marrow hematopoiesis, I mapped twelve AML samples from t (8; 21) subgroup and revealed an early myeloid pattern of aberrant differentiation associated with RUNX1-RUNX1T1 fusion. This key driver aberration initiates an early erythromyeloid (erythroid, megakaryocytic, and mast) priming and results in an enrichment of mast progenitors in t (8; 21) AML.

Conclusion: We identified three mast progenitor surface markers from our scRNAseq cohort and validated mast progenitor cell population by FACS in several AML samples. Mast progenitor in AML is a heterogenous population originated from the leukemic blasts. Variation in leukemic cell hierarchies result in mast progenitor as a heterogeneous population in AML. Further functional modeling is needed to validate malignant mast progenitor cellular origin predictions from single cell transcriptomics.