Cole Foundation
Research Celebration Day
18th Year

18e Année
Célébrons la recherche
de la Fondation Cole

INRS
Institut national de la recherche scientifique

CF

McGill

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 2024 Fellows, the Fellowship programme has supported more than 230 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 $14 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

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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 2024, 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

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Mme Emma Tibaldo //
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The Cole Foundation

in association with

Université de Montréal

McGill University

Institut national de la recherche scientifique INRS

Research Celebration Day

in support of pediatric and young adult leukemia and lymphoma research

FRIDAY, MAY 3RD, 2024

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 Catherine Goudie //
Research Institute of the McGill University Health Centre
Cancer Predisposition Syndromes in Children and Young Adults: Let's talk about "screening"

Dr Leandra Desjardins //
Research Centre of the Sainte-Justine University Hospital
Implementing transition readiness screening in pediatric oncology long-term follow-up care

Dr David Ogez //
Maisonneuve-Rosemont Hospital Research Center, Faculty of Medicine, Université de Montréal
Improving the quality of life of pediatric hemato-oncology patients through hypnotic communication and distraction techniques.

2:00 P.M. - 3:15 P.M.

Keynote speaker: Dr Ravi Majeti MD, PhD //
Stem Cells and Reprogramming in Human Acute Leukemia
Dr Ravi Majeti MD, PhD is the Virginia and D.K. Ludwig Professor, Professor of Medicine (Hematology), and Director of the Institute for Stem Cell Biology and Regenerative Medicine at the Stanford University School of Medicine. Dr Majeti is a board-certified hematologist, having completed his Hematology Fellowship at Stanford. While there, he investigated acute myeloid leukemia (AML) stem cells and therapeutic targeting with anti-CD47 antibodies. Dr Majeti directs an active NIH-funded laboratory that focuses on the molecular characterization and therapeutic targeting of leukemia stem cells in human hematologic disorders, particularly AML, and has published >120 peer-reviewed articles. He is a recipient of the Burroughs Wellcome Career Award for Medical Scientists, the New York Stem Cell Foundation Robertson Investigator Award, the Leukemia and Lymphoma Society Scholar Award, and the Clifford Prize. Dr Majeti is a member of the American Association for Cancer Research (AACR) Task Force on Hematologic Malignancies. He also serves on the editorial boards of Blood and Cancer Discovery.

A RECEPTION WILL FOLLOW

Address:
McIntyre Medical Building, 6th floor
Faculty of Medicine and Health Sciences McGill University
3655 Promenade Sir William Osler, Montreal, QC

The 2024 – 2026 Cole Foundation Fellows

MCGILL UNIVERSITY

Sarah Chehayeb //
PhD program

Supervisor: Sylvie Lambert

Project title: Co-design of a self-management intervention with young adults post allogenic bone marrow transplant, their caregivers, and the healthcare team: A feasibility study

Description: This study uses a co-design method to develop a self-management support intervention following an allogenic bone marrow transplant. We will hold workshops with adolescents and young adults diagnosed with leukemia or lymphoma, their caregivers, and the healthcare team. The feasibility and acceptability of the intervention will then be examined.

Bahareh Jafari //
PhD program

Supervisor: Kolja Eppert

Project title: Investigating the role of ASXL1 mutations on the development and progression of acute myeloid leukemia

Description: Mutated ASXL1 is one of the driver genes of acute myeloid leukemia. Through single-cell gene expression and functional analysis of mutated human HSCs, we will identify the mechanisms through which ASXL1 mutations alter human HSC function, leading to cancer.

Olivia Kovecses //
PhD program

Supervisors: Maureen McKeague & François Mercier

Project title: Developing Differentiation Regulators for Acute Myeloid Leukemia using RNA Therapeutics

Description: As acute myeloid leukemia (AML) remains challenging to cure, the project focuses on developing RNA therapeutics to target transcription factors that are often dysregulated in AML. By designing RNA duplexes to activate these targets, we aim to restore the expression of dysregulated genes and reprogram immature leukemic blasts to undergo terminal differentiation.

Mathieu Mancini //
Post-PhD program

Supervisor: David Langlais

Project title: Omics-based selection of potentiated cord blood-derived NK cells for acute myeloid leukemia therapy

Description: To tackle treatment of acute myeloid leukemia (AML), we propose to obtain cord blood NK cells and increase and prolong their tumor-killing capacity through small molecule PTPN1/2 inhibition. Since different donors exhibit different potentials for yielding high-quality therapeutic NK, we will perform single cell transcript and epitope sequencing (CITE-seq) on donor NK and identify key biomarkers that predict optimal cord blood donors.

UNIVERSITÉ DE MONTRÉAL

Jamie Beaulieu //
PhD program

Supervisor: David Knapp

Project title: Precise Human Leukocyte Antigen (HLA) gene editing to extend transplants to underserved patients

Description: Finding a suitable HLA-matched donor for hematopoietic stem cell transplant (HSCT) is rarely successful for Canadian leukemia patients who have an ethnic minority background. This project aims to develop a gene editing technology to specifically and permanently change the key HLA genes in candidate sources while also maintaining the key regenerative properties of hematopoietic stem cells (HSCs).

Pault Yeison Minaya Ferruzo //
PhD program

Supervisor: Frederick A. Mallette

Project title: Disrupting the Maintenance of Leukemia: Targeting Transcriptional and Translational Programs with KDM4A Inhibitors and Rocaglates

Description: Pediatric Acute Myeloid Leukemia (AML) resilience relies on the abnormal activation of cancer-supporting genes and the unchecked production of proteins that help cancer cells survive. Our project proposes that targeting the epigenetic circuitry and over-translation of survival proteins will disrupt essential processes unique to AML, potentially improving survival and treatment response.

Roudy Farah //
PhD program

Supervisor: Moutih Rafei

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

Description: In contrast to normal cells, leukemic and other cancer cells express cell surface proteins called cancer/testis antigens (CTAs). Our objective is to exploit this difference by engineering a mesenchymal stromal cell-based vaccine capable of stimulating a CTA-specific immune response that will be assessed using lymphoma/leukemia models in vivo.

Laurence Gagné //
Post-PhD program

Supervisor: Frédérick A Mallette

Project title: Role of Mutant IDH in The Maintenance of Telomeric Functions in Pediatric Acute Myeloid Leukemia

Description: Pediatric AML can arise from mutations in isocitrate dehydrogenases, producing the oncometabolite R-2-hydroxyglutarate (R-2HG), associated with a poorer prognosis. We propose to investigate R-2HG's role in telomere biology, potentially leading to the development of anticancer therapies and a deeper understanding of pathways promoting telomere-associated genomic instability.

Nafiseh Keshavarzian //
PhD program

Supervisors: Denis-Claude Roy and Vibhuti Dave

Project title: Developing TCR-T cell therapy approaches targeting MiHA in hematological malignancies

Description: This project will be to develop and optimize anti-MiHA TCR therapy for the treatment of hematological malignancies. We hypothesize that finding the most potent MiHA-TCR for a given MiHA, along with a competent cell to be transduced will arise into an auspicious cell therapy for patients with blood-related cancers.

Tyler Lussier //
PhD program

Supervisor: Brian Wilhelm

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

Description: Drug resistance in pediatric AML remains a problem with the role of the bone marrow microenvironment becoming increasingly appreciated. In this project, we will co-culture AML cells with HS-5, a human bone marrow stromal cell line, to identify AML surface proteins involved in drug resistance through a CRISPR-based screen.

Milad Ahmadi Najfabadi //
Post-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)

Project Title: Improving CAR T Cell Targeting in Hematological Malignancies: The Synergistic Impact of Rasal1 Downregulation and Hinge Modification in the Anti-CD19-scFv-CD28-CD3z CAR construct

Description: Chimeric antigen receptors have proven to be effective for immunotherapy against various cancer cells. We recently discovered a novel negative regulator of T-cell activation and function termed Rasal1 that associates with the antigen receptor on T-cells. My proposal aims to enhance the efficacy of CAR T cell therapy via the rational design of novel CARs with unique hinge regions combined with the use of CRISPR or shRNA methods to down-regulate Rasal1 to potentiate CAR targeting of Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).

Nadia Emely Chauca Torres //
PhD program

Supervisor: Sonia Cellot

Project Title: Deciphering JAK-STAT signaling in Acute Megakaryoblastic Leukemia

Description: Pediatric Acute Megakaryoblastic Leukemia (AMKL) constitutes up to 16% of AML cases with a typically poor prognosis.
Our project targets the hyperactive JAK-STAT pathway in AMKL to understand its role in disease progression. Through molecular and proteomic analyses in murine models, we aim to identify therapeutic targets for improved treatment strategies.

Research abstracts from current Cole Fellows

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

Auteur : Camille Aitchedji

Co-auteurs : Moison C. ; Fortier S. ; Spinella J.F. ; Gracias D ; et Sauvageau G.

Affiliation: Guy Sauvageau, Laboratoire de génétique moléculaire des cellules souches, Institut de recherche en immunologie et en cancérologie (IRIC)

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

Introduction : 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).

But de l'étude : 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.

Méthodes : 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 exposé 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.

Résultats : Le screen CRISPR/Cas9 a identifié une cible moléculaire potentielle impliquée dans la régulation de l'expression des gènes au niveau transcriptionnel. L'inactivation par shRNA et sgRNA de cette protéine kinase a démontré la perte d'activité des composés du CCC163 suggérant qu'elle est la cible de ces composés. Des essais *in vitro* ont ainsi confirmés l'inhibition de l'activité catalytique de cette kinase par les molécules du CCC163. D'autre part, l'analyse du transcriptome a révélé une diminution de l'expression de gènes connus pour être régulés par la cible identifiée du CCC163. Une voie de signalisation impliquant des mécanismes épigénétiques pouvant engendrer une reprogrammation des lignées LMA a également été mis en évidence en présence de nos composés. Finalement, nous avons déterminé que la sensibilité au CCC163 est associée à des mutations sur *FLT3* alors que les mutations sur *TP53* sont associés à une résistance, dans nos 124 spécimens de LMA.

Conclusion : Ce projet a permis d'identifier une protéine kinase comme 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: CAR-NK cell immunotherapy against severe SARS-CoV-2 for immunocompromised pediatric HSCT recipients

Author: Mila Bjelica

Co-Authors: Hugo Roméro, Tram NQ Pham, Kathie Béland, Anne Duchesne, Marilou Henri, Véronique Lisi, Vincent-Philippe Lavallée, Martin Smith, Étienne Gagnon, Andrés Finzi, Éric A Cohen, Élie Haddad

Affiliation: Dr Elie Haddad, Pediatrics, CHU Sainte-Justine and Dr Etienne Gagnon, Microbiology, Infectiology and Immunology, University of Montreal

Key words: Immunotherapy, infectious disease, NK cells, post-HSCT treatment, chimeric antigen receptor (CAR)

Background: The leading therapy for high-risk/relapsed pediatric leukemia are hematopoietic stem cell transplants (HSCT). However, HSCT recipients are at a high risk of infections by pathogens that are relatively controlled in otherwise healthy people. This includes SARS-CoV-2, with a 6% mortality rate in children having received a HSCT. Natural killer (NK) cells are critical in managing SARS-CoV-2 infection. NK cell therapies are relatively safe, showing a lower risk of Graft versus Host Disease, and no risk of cytokine storm. Moreover, NK therapies can be produced for off-the-shelf use, to be available as soon as they are needed, since NK transfer does not require a matching donor. Therefore, we propose to develop chimeric antigen receptor (CAR) NK cells specific against SARS-CoV-2 to combat severe COVID19 in vulnerable patients.

Purpose: We aim to produce anti-SARS-CoV-2 CAR-NKs and to assess their efficacy and specificity in cell and mouse models.

Methods: CAR constructs included ScFV sequences derived from a pediatric COVID19 patient antibody, fused to KIRS2/DAP12 signalling domains. NK cells were expanded from the peripheral blood of healthy adults using K562 mbIL-21 feeders, depleted of $CD3^{+}$ cells and transduced with a baboon envelop pseudotyped lentivirus vector coding for our CAR constructs. The efficacy of the CAR-NKs was tested by cytotoxic assays against 697 expressing spike and CALU-3 infected with SARS-CoV-2.

Preliminary results: CAR-NK cells had superior lysis of spike^{+} targets, than spike^{-} targets: 34.17%-46.37% increase in lysis, depending on E:T ratio ($p<0.001$, $n=3$). CAR-NKs improved cytotoxicity compared to UT NKs: 37.62%-52.92% increase in specific lysis, depending on E:T ratio ($p<0.001$, $n=3$). Preliminary data suggests CAR NK are also effective and specific when tested against CALU-3 SARS-CoV-2^{+}.

Conclusion: Preliminary results suggest our anti-spike CAR-NK cells against SARS-CoV-2 are target specific and have a more potent antiviral effect than untransduced NK cells. This may serve as a proof of concept for CAR-NKs in treating severe opportunistic infections in children with cancer, but further testing is needed. Ultimately, we hope to develop off-the-shelf immunotherapies against viral diseases that cause significant complications in children treated for blood cancers, which are effective with minimal toxic side effects.

Title: Characterization of human HLA-DR+ NK cells

Author: Capucine Bourel

Affiliation: Dre Sylvie Lesage, Hôpital Maisonneuve-Rosemont / Université de Montréal

Keywords: NK cell, HLA-DR, human, leukemia, cell therapy

Background information: Natural Killer (NK) cells are cytotoxic cells capable of efficiently recognizing and eliminating leukemic cells. Indeed, high numbers of circulating NK cells are associated with a better prognosis for patients with acute myeloid leukemia (AML) and a reduced risk of relapse in patients with chronic myeloid leukemia (CML). Before becoming mature NK cells (mNK), NK cells go through various stages of development. In mice, one of these stages includes pre-mNK cells, which are precursors of mNK cells. Like mNK cells, pre-mNK cells produce effector cytokines (IFN-g and TNF-a) and display cytotoxic activity. In addition, pre-mNK cells generate mNK cells with very high anti-tumour activity. These characteristics make pre-mNK cells a highly attractive cell population for the development of anti-cancer therapies. However, the equivalent of pre-mNK cells remains to be identified in humans. Studies have shown that human HLA-DR+ NK cells possess characteristics similar to those of murine pre-mNK cells, such as a high production of IFN-g. Additionally, a high proportion of HLA-DR+ NK cells correlate with a better prognosis in many human cancers.

Purpose of the study: We hypothesize that human HLA-DR+ NK cells are equivalent to murine pre-mNK cells and generate mature NK cells with high anti-tumour activity. We aim to define the similarities between human HLA-DR+ NK cells and murine pre-mNK cells, as well as their capacity to generate mature NK cells with high anti-tumour potential.

Methods:

#1. To determine the ability of human HLA-DR+ NK cells to generate mNK cells in vivo, we use the NSG-HuIL15 mouse model, which lack T, B and NK cells and are transgenic for human IL-15. HLA-DR+ NK cells will be isolated from the PBMC of healthy donors and injected in the mice. We will analyze the maturation, phenotype, and function of these cells 1 month after transfer. By flow cytometry, we will determine whether they have acquired a mature phenotype ($CD16^{+}CD56^{dim}CD57^{+}$). We will also compare this phenotype to mNK cells generated from HLA-DR- NK cell precursors. The expression of cytokines and cytotoxic proteins

will also be quantified. To test for the cytotoxic potential, we will use the K562 cells as targets, which are derived from a patient with chronic myeloid leukemia. We will compare the cytotoxic and antitumour potential of HLA-DR$^+$ NK cells with that of HLA-DR$^-$ NK cells in vitro and in vivo in NSG-HuIL15 mice.

#2. To define the similarities between human and murine NK cells, and to assess the heterogeneity of the different NK cell populations, we will compare the transcriptome of human NK and murine NK cells using scRNASeq. Unbiased analysis will reveal the similarities between mouse pre-mNK cells and human HLA-DR$^+$ NK cells.

Results: Similar to murine pre-mNK cells, we find that HLA-DR$^+$ NK cells express markers of immature NK cells, consistent with a precursor phenotype. In addition, we have successfully optimized a protocol for the transfer and survival of low number of human NK cells into mice, which will enable the study of human NK cell maturation in vivo. We have also developed an efficient in vitro expansion protocol for human NK cells.

Conclusions: We expect that mature NK cells derived from HLA-DR$^+$ NK cells will have greater anti-tumour potential than cells derived from HLA-DR$^-$ NK cells. Although we expect to observe a significant species-related difference, scRNAseq data will reveal common surface markers or transcription factors between mouse pre-mNK and human HLA-DR$^+$ NK cells, facilitating the characterization of these cells in vivo. The characterization of HLA-DR$^+$ NK cells, which are highly proliferative and produce significant amounts of pro-inflammatory cytokines will provide potential new avenues for the development of therapies targeting leukemias.

Title: Characterization of metabolic rewiring and identification of potential therapeutic targets in CBFA2T3-GLIS2-dependent AMKL

Author: Caroline Capdevielle

Affiliation: Laura Hulea and Frederick A. Mallette, Division of Immunology-Oncology, Maisonneuve-Rosemont Hospital Research Centre; Department of Biochemistry and Molecular Medicine, Université de Montréal.

Keywords: AMKL, CBFA2T3-GLIS2, metabolomics, metabolic vulnerabilities, interactome

Background information: Acute megakaryoblastic leukemia (AMKL) represents ~10% of pediatric acute myeloid leukemias and is associated with extremely poor prognosis. Importantly, the *CBFA2T3-GLIS2* (CG2) gene fusion is the most observed lesion amongst all pediatric AMKL patients (~20%), is associated with chemotherapy resistance and shows the poorest prognosis (<20%; 5-year survival) compared to the other gene fusions. Several studies demonstrated the importance of metabolic adaptation for the survival of acute myeloid leukemia cells, but also the therapeutic potential of targeting metabolic vulnerabilities. However, the potential role of *CG2* fusion and its interactors in causing metabolic alterations in AMKL remains unexplored.

Purpose of the study: We propose to investigate how metabolic rewiring associated to *CG2* gene fusions and interactors contribute to malignant transformation in order to identify and validate new metabolic therapeutic target.

Methods: Using mouse hematopoietic stem and progenitor cells (HSPCs) expressing *CG2* gene fusion, metabolic gene expressions were analyzed by RNA-seq associated with GC-MS analysis to quantify metabolite levels. In parallel, inhibition of GLIS2 expression/activity by CRISPRcas9 was performed in *CG2*-positive human AMKL cell line to characterize metabolic changes by RNA-seq, Seahorse or GC-MS analysis. To identify therapeutic vulnerabilities in AMKL cell lines, shRNA vectors against *PC, PDK2* or the pharmacological inhibitor AZD7545 were used, and cell proliferation was measured after 7 days of treatment. Finally, to identify the partners of *CG2* fusion proteins and their role in AMKL, we performed a protein proximity-dependent biotin labeling (BioID) coupled with mass spectrometry in M07e cell line.

Results: Transcriptomic analyses in *CG2*-driven mouse AMKL cells identified genes pertaining to pyruvate metabolism, such as pyruvate dehydrogenase kinase 2 (*PDK2*) and pyruvate carboxylase (*PC*). Further investigations showed that inhibition of *GLIS2* by CRISPR-Cas9 alters mitochondrial respiration and glycolysis. Based on transcriptomic analyses, we decided to target *PC* and *PDK2*, to elucidate their functions in AMKL cell line. Using shRNA, we observed a decrease in proliferation 7 days post-infection. In addition, treatment with AZD7545, an inhibitor of PDK activity, has a strong effect on proliferation in *CG2*-positive AMKL but also in patient-derived xenograft *in vitro.* In parallel, we identified several partners of transcriptional regulations, suggesting that *CG2* metabolic regulations might occur via gene transcription.

Conclusion: In conclusion, we propose that *CG2* induces metabolic rewiring of hematopoietic cells to promote leukemogenesis. Altered metabolism of cancer cells could create metabolic vulnerabilities that can be targeted with pharmacological inhibitors of metabolic enzymes. None of these vulnerabilities have been previously described in AMKL cell lines and represent novel potential therapeutic targets. Using murine models, in combination with patient-derived cell lines, we seek to identify and validate therapeutic metabolic targets for *CG2*-positive pediatric AMKL. All these experiments will directly support the advancement of optimized clinical protocol that have proven unsuccessful in children with AMKL.

Title: Near-perfect precise on-target editing of human hematopoietic stem and progenitor cells

Authors: Fanny-Meï Cloarec-Ung, Jamie Beaulieu, Arunan Suthananthan, Bernhard Lehnertz, Guy Sauvageau, Hilary M Sheppard, David JHF 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. Precision edits can be generated using a CRISPR/Cas system to introduce a break at the target locus together with the addition of a template DNA to engage the homology-directed repair (HDR) pathway and insert the edit of interest. These templates contain the change of interest flanked by homology arms matching the sequence on either side of the break site. While this strategy can function, non-homologous end joining (NHEJ) which results in random insertions and deletions (indels) is the default pathway, as HDR can only operate in the S and G2 phase of the cell cycle. As such, the efficiency of HDR mediated editing, particularly in relevant primary human stem and progenitor populations remains limited, with most strategies achieving efficiencies in the range of 10-20%. Despite such limited efficiencies, multiple clinical trials using CRISPR/ Cas-based editing strategies are currently ongoing, reinforcing the extreme interest in the area.

Purpose of the study: In this project, we aim to use the optimised CRISPR editing system to study the mutation RUNX1 among other mutations in the context of leukemia.

Methods / Results: Through an optimization of guide RNA delivery, donor design, and additives, we have now obtained mean precise editing efficiencies >90% on primary cord blood HSCPs with minimal toxicity and without observed off-target editing. The main protocol modifications needed to achieve such high efficiencies were the addition of the DNA-PK inhibitor

AZD7648, and the inclusion of spacer-breaking silent mutations in the donor in addition to mutations disrupting the PAM sequence. Critically, editing was even across the progenitor hierarchy, did not substantially distort the hierarchy or affect lineage outputs in colony-forming cell assays or the frequency of high self-renewal potential long-term culture initiating cells. As modelling of many diseases requires heterozygosity, we also demonstrated that the overall editing and zygosity can be tuned by adding in defined mixtures of mutant and wild-type donor.

Conclusion: With these optimizations, editing at near-perfect efficiency can now be accomplished directly in human HSPCs. This will open new avenues in both therapeutic strategies and disease modelling, especially in the study of the mutation RUNX1.

Title: Characterization of KMT2A-rearrangmeent Acute Myeloid Leukemia biomarkers

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 of the study: Methyltransferase-like 7B (METTL7B), a biomarker we identified but not yet characterized, was found to influence proliferation in preliminary testing of the biomarkers in KMT2A-MLLT3 cell lines and was thus chosen to be one of the proteins of interest for further characterization of its transcription regulation and protein activity within KMT2A-MLLT3 leukemic cells.

Methods: Using human acute myeloid leukemia cells expressing the KMT2A-MLLT3 fusion, an inducible-shutoff system for *METTL7B* was generated. With these cells, gene expression was analyzed to identify differentially expressed genes dependent on the expression of *METTL7B*. In parallel, protein interactors of METTL7B were identified by protein proximity-dependent biotin labeling (BioID) in four KMT2A-MLLT3 AML cell lines (completed in technical triplicate), with those consistently interacting with the bait protein recognized and their activities compared to known effects of METTL7B expression. Finally, live cell and fixed cell microscopy were completed to validate the localization of METTL7B in KMT2A-MLLT3 AML cells to identify potential functions within the cell.

Results: Transcriptomic analyses in the KMT2A-MLLT3 *METTL7B*-shutoff cells identified genes pertaining to amino acid biosynthesis to be significantly upregulated when *METTL7B* expression is lost, including asparagine synthase (*ASNS*) and Cystathionine Gamma-Lyase (*CTH*) which can play a role in cell cycling

and methylation, respectively. Further investigations showed that inhibition of *METTL7B* was correlated with an increase in expression of its closest relative in the methyltransferase-like protein family, *METTL7A*. Both METTL7B and METTL7A have been shown to have the methyltransferase ability for alkyl and phenolic thiol-containing substrates in recent literature. 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 METTL7B, similarly as described in multiple solid cancers. Microscopy images of METTL7B's localization experiments within KMT2A-MLLT3 AML cells supports its activity in the cytoplasm, specifically in the endoplasmic reticulum.

Conclusion: In conclusion, we propose that METTL7B's activity as a methyltransferase induces several effects in KMT2A-MLLT3 AML cells that may promote or support leukemogenesis. The fact that METTL7B 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 METTL7B have previously been described, however with METTL7B's rising interest as a putative oncogene and biomarker in both liquid and solid cancers, this would represent a novel potential therapeutic target.

Title: Engineering of natural killer cell against therapy-induced senescent cells

Authors: Joshua Dulong[1,2], Louise Rethacker[2,3], Marie-Ève Lalonde[4], Georgio Mansour Nehmo[1,2], Basma Benabdallah[2], Romain Gioia[2], Kathie Béland[2], Richard Marcotte[3,4], Elie Haddad[2,3,5] and Christian Beauséjour[1,2]

Affiliations: [1]Département de Pharmacologie et Physiologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada

[2]Centre de Recherche du CHU Ste-Justine, Montréal, Québec, Canada

[3]Département de Microbiologie, Infectiologie et Immunologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada

[4]National Research Council Canada, 6100 Royalmount Avenue, Montreal, QC H4P 2R2 Canada

[5]Département de Pédiatrie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada

Keywords: Senescence, Immunotherapy, NK cells, CRISPR screen, iPSC

Background: Cancer treatments (chemotherapy and radiotherapy) or leukemic cells themselves were shown to induce senescence in the bone marrow microenvironment. These senescent cells are non-proliferative but remain metabolically active and can interact with their environment through their secretion phenotype. Although senescent cells can be eliminated by the immune system, including natural killer (NK) cells, it has become evident that a subpopulation employs diverse mechanisms to evade clearance. The accumulation of senescent cells can be deleterious as they enhance cancer cell proliferation and recruitment of immunosuppressive cells (i.e. monocytes). Previous studies showed that senescent fibroblasts can escape NK cell clearance by inducing the expression of the ligand HLA-E and the ganglioside GD3.

Purpose of the study: NK cells are of high interest in immunotherapy since they typically do not induce graft-versus-host disease, making them the ideal off-the-shelf therapy to target senescent cells. Thus, the aim of this study is to identify immune evasion mechanisms of senescent cells in order to engineer more efficient NK cells.

Methods: To overcome the resistance induced by the binding of HLA-E to its receptor (NKG2A) and the binding of sialic acids present on GD3 to the Siglec-7 receptor, we generated (1) NKG2A knockout (KO) iPSC and NK cells using CRISPR technology, and (2) treated senescent cells with sialidase or used neutralizing antibody targeting Siglec-7. We also investigated the impact of senescence environment on the sensitivity of cancer cell lines (HT29, MCF7, A549, NALM6 and 697) to NK cells.

Results: We first measured the ability of our NKG2A KO NK cells to lyse fibroblasts overexpressing artificially HLA-E. While our engineered NK cells were capable of efficiently lysing these fibroblasts, they were not more competent at lysing ionizing radiation-induced senescent fibroblasts. Likewise, sialidase treatment and neutralizing antibody targeting Siglec-7 could not increase the ability of NK cells at lysing senescent fibroblasts, suggesting that other resistance mechanisms are involved. Moreover, we confirmed that senescent environment can either protect or sensitize cancer cells to NK cell lysis.

Conclusion: We are currently planning a whole-genome CRISPR screen in senescent fibroblasts and in NK cells to identify new mechanisms of immune evasion. Because it is possible to generate multiple genetic alterations at the iPSC level, our study should lead to the development of an ultimate off-the-self senolytic iPSC-derived NK cell therapy.

Titre : Caractérisation du rôle d'eIF4A dans la résistance des cellules de la leucémie myéloïde aiguë à la chimiothérapie et aux thérapies ciblées

Auteurs et affiliation : Victor Gife[1,2], Katie Fooks[3,4], Alejandro Schcolnik-Cabrera[1,2], Raquel Aloyz[3,4], Laura Hulea[1,2*] and François Mercier[3,4*]

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

Mots clés : Leucémie aiguë myéloïde (LAM); résistance; chimiothérapie; traduction; métabolisme.

Informations générales :
La leucémie aigüe myéloïde (LAM) est un cancer affectant les cellules progénitrices hématopoïétiques de la lignée myéloïde, les poussant à une prolifération et une différenciation anormale. Les seuls traitements actuels disponibles sont des chimiothérapies myéloablatives pour les patients sans comorbidités, comme la combinaison de cytarabine et daunorubicine. Cependant, la LAM rechute dans plus de la moitié des patients traités avec chimiothérapie et il y apparaît une résistance à ces traitements.

Cette résistance aux traitements a été associée en partie avec une reprogrammation métabolique, qui pourrait s'expliquer par un changement de l'expression génique au niveau transcriptionnel/post-transcriptionnel apportant une plasticité métabolique (phosphorylation oxydative, glycolyse). Des études précédentes ont montré une réactivation de la voie mTORC1 suite aux traitements à la cytarabine, dans un modèle murin *in vivo* de LAM (1). La dérégulation de la traduction de l'ARNm est reconnue dans des nombreux cancers. eIF4F (eukaryotic initiation factor 4F) est un complexe protéique (composé de eIF4A, eIF4E et eIF4G), sous le contrôle de mTORC1, qui régule directement l'initiation de la traduction. eIF4A, la protéine hélicase de ce complexe, représente un intérêt particulier comme cible potentielle pour combattre la résistance aux traitements dans la LAM.

Méthodes : Nous proposons que les cellules leucémiques exposées à une chimiothérapie activent plusieurs voies métaboliques contrôlées par la traduction afin de s'adapter aux traitements.

Résultats et conclusions : Nos données préliminaires montrent que l'inhibition spécifique de eIF4A avec des petites molécules (rocaglates) tuent spécifiquement les cellules LAM résistantes à la cytarabine *in vitro* et *in vivo*, et affectent le métabolisme bioénergétique et l'équilibre de métabolites intracellulaires (2). De plus, nous montrons qu'une combinaison de traitement semble possible avec le vénétoclax, un inhibiteur spécifique de la protéine anti-apoptotique BCL2, déjà utilisé dans le traitement de la LAM et dans le cas de rechute.

(1) Oki, Toshihiko et al. "Imaging dynamic mTORC1 pathway activity in vivo reveals marked shifts that support time-specific inhibitor therapy in AML." *Nature communications* vol. 12,1 245. 11 Jan. 2021, doi:10.1038/s41467-020-20491-8

(2) Fooks K, Galicia-Vazquez G, **Gife V**, et al. EIF4A inhibition targets bioenergetic homeostasis in AML MOLM-14 cells in vitro and in vivo and synergizes with cytarabine and venetoclax. *J Exp Clin Cancer Res*. 2022;41(1):340. Published 2022 Dec 9. doi:10.1186/s13046-022-02542-8

Title: FLT4 receptor deregulation promotes leukemia survival and chemotherapy resistance

Author: Djazia Haferssas

Affiliation: Dr Casimiro Gerarduzzi, Department of pharmacology and physiology, Université de Montréal ; Centre de Recherche de l'Hôpital Maisonneuve Rosemont (CR-HMR)

Keywords: RTK, MDM2, MDMX, p53, chemoresistance

Background information: Aberrant Receptor Tyrosine Kinase (RTK) signaling allows cancer cells to modulate survival, proliferation, and death, leading to tumorigenesis and chemoresistance. In leukemia, one of the frequently deregulated receptors is FLT4 (VEGFR-3), which correlates with the progression of leukemia. However, little is understood about its overexpression and association with drug resistance. Moreover, chemotherapies require the activation of wild type p53 (wt) in tumor cells to induce their apoptosis. Although p53 is rarely mutated in leukemia (5%), it is not understood why its wild type state is non-functional. Indeed, p53 is negatively regulated by the MDM2/MDMX complex, which is overexpressed in leukemia, potentially leading to p53 inactivation. The extracellular signals modulating the MDM2/MDMX complex levels and, therefore, p53 could come from deregulated RTKs to increase the survival of tumor cells.

Purpose of the study: characterize the mechanisms of p53 inactivation by FLT4 in leukemic cells to re-activate p53 function and sensitize leukemic cells to chemotherapy-induced apoptosis.

Methods: Using HEK293T and U2OS cellular models, FLT4, MDM2 and MDMX vectors were co-transfected to assess the stability and localization of MDM2/MDMX. The MDM2/MDMX complex was immunoprecipitated and analyzed by Mass Spectrometry to determine the protein network modification under FLT4 activation. Second, the ALL leukemic cell line (REH) was **1)** treated with the FLT4 specific ligand (VEGF-C) or **2)** transduced with a lentiviral vector encoding for FLT4 to induce its constitutive activation and was studied for MDM2/ MDMX stability, p53 expression, survival under chemotherapy treatment (Doxorubicin) and proliferation. Finally, leukemic cells transduced with Luciferase and FLT4 vectors were injected in immunodeficient mice. The leukemic cells proliferation and metastatic potential were monitored by In vivo Imaging System (IVIS).

Results: The activation of FLT4 stabilizes the complex MDM2/ MDMX. Immunoprecipitation of the heterodimer complex revealed a consensus sequence of CDK4/6 that was phosphorylated when FLT4 was overexpressed, which we confirmed was mediated by CDK4/6 and necessary for MDM2/MDMX stability. The increase of the MDM2/ MDMX complex levels leads to the ubiquitin-induced degradation of p53 and reduction of the expression of its target genes implicated in apoptosis. In addition, FLT4 promotes the relocalization of the complex MDM2/MDMX into the cytoplasm, limiting p53 transcriptional activity in the nucleus. The activation of FLT4 in the leukemic cells (ligand-dependant or constitutive activation) leads to p53 suppression associated with resistance to chemotherapy-induced apoptosis and increased proliferation. Finally, the leukemic cells overexpressing FLT4 showed higher proliferation and increased metastatic potential to distant organs impacting therefore mice survival.

Conclusion: Acute lymphoid leukemia is characterized by the FLT4/ VEGF-C signaling pathway of MDMX/MDM2/ p53 axis deregulation. The aberrant activation of FLT4 increases the proliferation and survival of leukemic cells by stabilizing the complex MDM2/MDMX and suppressing p53-mediated cell death.

Title: Multi-function T cells for precision Immunotherapy

Author: Leila Jafarzadeh

Affiliation: Jean-Sebastien Delisle lab, Division of Immunology-Oncology, Maisonneuve-Rosemont Hospital Research Centre; Université de Montréal.

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

Background information: Allogeneic Hematopoietic Cell Transplantation (AHCT), a potent treatment for blood cancers, is hindered by high relapse rates, graft-versus-host disease (GVHD), and immunosuppression complications, including viral reactivations. To mitigate GVHD, a common regimen includes early pharmacological immunosuppression followed by a calcineurin inhibitor like Tacrolimus (or FK506), which requires binding to the cytosolic protein FKBP12 to exert its effect. However, Tacrolimus inhibition could compromise targeted cellular therapeutics against viruses and malignant cells post-transplant. The administration of anti-CD19 CAR T cells generated from poly-specific T-cell repertoires post-AHCT has shown promise treat relapsing B-cell neoplasms post-transplant but are associated with GVHD while virus-specific T cells are innocuous but subject to inhibition by immunosuppressive drugs like tacrolimus. A single-cell product combining tacrolimus resistance, virus specificity, and CAR expression could address immune deficiemncies post-AHCT without causing GVHD.

Purpose of the study: This project aims to produce and characterize Tacrolimus-resistant virus-specific T cells (VSTs) expressing anti-CD19 CAR through FKBP12 knockout, to improve treatment outcomes for patients with blood cancers post-AHCT.

Methods: PBMCs from healthy donors were stimulated with anti-CD3 and anti-CD28 antibodies (polyclonal activation). The FKBP12 knockout was performed by nucleofection of Cas9 small-guide (sg)RNA ribonucleoprotein (RNP), followed by CD19-CAR transduction with lentivirus two days after stimulation. Edited (FKBP12KO) and unedited (Cas9) T/CAR T cells were divided into Tacrolimus-treated and untreated groups. Editing success was assessed using tracking of indels by decomposition (TIDE) sequencing and protein loss via Western blot. End-of-culture assessments included cell counts, CAR transduction efficiency (CD271 reporter), and phenotypic analysis for memory/

effector subsets (CD45RA and CCR7) and T-cell dysfunction markers (PD-1, TIM-3, 2B4, LAG3). Functional assays included cytokine production (intracellular flow cytometry for IFN- , TNF- and IL-2) and cytotoxicity. Anti-CD19 cytotoxicity was conducted using NALM6 cells (CD19-expressing) as targets and CD19-negative Jurkat cells as controls.

Results: Our study confirms the efficacy of FKBP12 knockout in T and CAR T cells, showing a T effector memory (Tem) phenotype. Tacrolimus treatment was not associated with significant changes in cell growth but, treated FKBP12 KO T cells displayed significant IFN-g and TNF-a production compared to treated Cas9 T cells. Similar trends were observed in CD19-CAR T cells, regardless of treatment concerning differentiation. However, LAG3 emerged as a prominent exhaustion marker in treated gene-knockout CAR T cells, displaying significantly higher expression levels. Functionally, IL-2 and TNF-a production were preserved in FKBP12 KO CAR T cells exposed to Tacrolimus while unedited CAR T cells were potently suppressed by tacrolimus. All CAR T cells maintained robust cytotoxicity, showing specific antigen-dependent killing, with no significant differences between edited and unedited CAR T cells in both treated and untreated conditions.

Conclusion: Our findings validate the integrity of our production process, indicating that FKBP12 gene knockout does not compromise the phenotype or function of T/CAR T cells and renders cells resistant to Tacrolimus. Subsequent investigations will explore CAR T-cell exhaustion/dysfunction through stimulation with multi-virus peptides and assess the expansion of antigen-specific FKBP12 KO CAR T cells under both Tacrolimus-treated and untreated conditions.

Title: TCR-T cells targeting Minor Histocompatibility antigens for the Treatment of Hematological Malignancies

Author: Nafiseh Keshavarzian

Affliliation: Annabelle Minguy, Nasser Masroori, Jean-Philippe Bastien, Jessica Trottier, Jaime Sanchez Dardon, Vibhuti Dave, Denis-Claude Roy, Division of Molecular Biology, Universite de Montreal Faculte de Medecine, Montreal, QC, Canada.

Keywords: TCR engineering, MiHA, hematological malignancies, T cell therapy.

Background: Allogeneic hematopoietic stem cell transplantation (aHSCT) remains the standard of care treatment for patients with high-risk or relapsing hematologic malignancies. Donor T cells present in the graft are crucial in mediating the potent graft-versus-leukemia (GVL) effect through the recognition of minor histocompatibility antigen (MiHA) mismatches expressed on cancer cells. However, donor T cells may also recognize MiHAs expressed in healthy tissues resulting in detrimental graft-versus-host-disease (GVHD). Therefore, isolating beneficial GVL activity from detrimental GVHD activity of donor T cells has significant potential in improving the aHSCT. Cellular therapy consisting of ex-vivo expansion of T cells recognizing MiHAs preferentially expressed on hematopoietic cells has shown great promise to maximize the GVL effect for hematological malignancies.

Purpose of study: The expansion of anti-MiHA T cells is challenging due to their low frequency in donor blood circulation. Engineering T cells to express MiHA-specific TCR will overcome this crucial issue. To this end, we expanded MiHA-specific T cells ex vivo, and isolated and cloned TCR sequences specific for several MiHAs from the expanded T cells. Here, we present the phenotypic and functional characterization of engineered T cells expressing individual TCR specific for two different MiHAs presented by HLA-B07.

Methods: We engineered primary T cells isolated from two donors to express two individual MiHA-specific TCRs and evaluated their phenotype and function. The engineered T cells were re-stimulated with APCs pulsed with cognate MiHA and T cell functionality was assessed by (a) production of IFN-g and TNF-a (b) expression of degranulation marker CD107a and (c) their capacity to eliminate MiHA-expressing DCs or target cells.

Result: Engineered T cells responded to both MiHA pulsed DCs and target cells with the former showing stronger response. Interestingly, we found that T cells from one donor consistently showed greater cytokine secretion, CD107a degranulation, and were more cytotoxic compared to T cells derived from the second donor. This is was observed for both MiHA-specific TCRs. Further, comparing response of T cells isolated from the same donor and engineered to express individual MiHA-specific TCRs restricted to the same HLA showed differences in their functionality likely reflecting differences in their affinity for their cognate MiHAs.

Conclusion: Taken together, these results indicate that the efficacy of engineered T cells is influenced by several parameters including the source of T cells to be engineered and the nature of TCR itself. Investigating these differences will allow us to select the optimal donors and TCRs to generate highly potent MiHA-specific TCR-T cells for the treatment of hematological cancers.

Title: Artificial neural network (ANN) classifier for automatic and fast annotation of acute myeloid leukemia cells

Author: Banafsheh Khakipoor, Véronique Lisi, Azer Farah, Vincent-Philippe Lavallée

Affiliation: Vincent-Philippe Lavallée, CHU Sainte-Justine Research Center/UdeM

Keywords: Bone Marrow, Acute Myeloid Leukemia, single-cell RNA sequencing, Machine Learning, Artificial Neural Network

Background: Acute myeloid leukemia (AML) is an aggressive cancer of hematopoietic stem/progenitor cells originating in the bone marrow. Leukemic cells and their microenvironment are diverse, vary across patients, and may be clinically quite relevant. Single-cell RNA sequencing (scRNAseq) is increasingly used to unbiasedly characterize patient-derived AML cells to address a variety of biological questions.

Purpose of study: Annotations of these cells rely on expert-based curations which are time-consuming and not generalizable. Additionally, AML cells are continuous and could be a mixture of categories, making it difficult to annotate specifically in-between cell types. There is thus an important need for an accurate and automated method to annotate the diverse cell types of normal hematopoietic cells and in AML. We hypothesize that we can develop a machine learning based classifier to automatically identify and annotate these cells.

Methods: We tested different machine learning algorithms using re-processed raw data from Human Cell Atlas bone marrow scRNA-seq dataset (8 donors, 250K cells). An extensive annotation effort was deployed using two previous annotations that were released for this dataset (each with strengths and limitations) and expert curation by our group. A subset of the reannotated cohort was used for training and validation.

Result: Artificial neural network (ANN) offered excellent accuracy and was selected, retrained and applied to AML datasets. Its performance was validated on an independent AML dataset comprising 15K curated AML cells (Van Galen et al, Cell 2019). We then applied the classifier on our current dataset of AML single-cell transcriptomes (Leucegene cohort). Over 940,000 cells from 200 patients were annotated in 5 seconds per sample. Marker analysis in individual samples and at the cohort levels supported the excellent performance of the algorithm.

Conclusion: In conclusion, we provide an accurate, fast, and now publicly available (github.com/lavalleelab) neural network-based cell-type classification algorithm for AML cells. Importantly, users don't need to provide an annotated reference, as with other published algorithms (e.g., Seurat Label transfer). Our classifier will enable homogeneous annotations to compare cell type abundances across AML samples, and uncover phenotypic differences in most similar cells across various conditions and genotypes.

Title: Development of CAR-NK Immunotherapy Using Gene-Edited Induced Human Pluripotent Stem Cells

Author: Alice MacDonald

Affliliation: Dr Elie Haddad, Department of Microbiology, Infectiology and Immunology, University of Montreal, CHU Sainte-Justine research

Keywords: Immunotherapy; Natural killer lymphocytes (NK); Induced pluripotent stem cells (iPSCs); Chimeric antigen receptors (CAR); CRISPR

Background: Autologous CAR-T cell therapy has achieved unprecedented success in treating blood cancer. However, there is a need for an "off-the-shelf" cell product that can be manufactured on a large scale and administered on demand. Natural Killer (NK) cells are innate lymphocytes with potent anti-tumor activity. Since they have MHC-independent cytotoxicity, allogeneic transplantation of NK cells is safe. However, immunosuppressive mechanisms in the tumor microenvironment can hinder NK cell anti-tumor function. Although genome editing of NK cells is a powerful tool for overcoming immunosuppression, technical challenges associated with NK cell gene editing have hampered the use of this approach in the development of immunotherapies. In this regard, induced pluripotent stem cells (iPSCs) can serve as a standardized and inexhaustible source of CAR-NK therapeutics cells, as they can be readily genetically modified and differentiated *in vitro* into NK cells.

Purpose of study: To develop CAR-NK lymphocytes derived from iPSCs (CAR-iNK) with enhanced anti-tumor activity, we aim to generate iPSC deficient for negative regulators of NK cell cytotoxicity, concomitant with the expression of a CAR before their *in vitro* differentiation into NK cells.

Method: We will target negative regulators of NK cell cytotoxicity by CRISPR to generate $iPSC^{KO}$ and we will use lentiviral transduction to induce anti-CD22 CAR transgene expression in modified iPSCs ($CAR\text{-}iPSC^{KO}$). Subsequently, CAR-iNK cells will be generated through cytokines directed *in vitro* differentiation and expanded using feeder cells. Their anti-tumor activity will be assessed *in vitro* against different leukemia cell lines; K562, NALM6, 697, and REH. Finally, we will establish proof of concept for our approach by testing the antitumor activity of CAR-iNK cells *in vivo* in a xenogeneic mouse model.

Results: We evaluated the feasibility of our approach in primary NK cells by using CRISPR to target *KLRC1*, which encodes NKG2A, a potent negative regulator of NK cells anti-tumor activity. Our findings confirm the relevance of targeting NKG2A, as *KLRC1*^{KO} NK cells demonstrate enhanced anti-tumor activity against four types of cancers *in vitro*. When adoptively transferred to a xenogeneic mouse model of metastatic cancer, *KLRC1*^{KO} NK cells significantly delayed tumor progression and prolonged survival compared to wild-type NK cells ($p=0.0015$). We generated both *KLRC1*^{KO} and anti-CD22 CAR iPSCs. Moreover, we were able to generate mature NK cells from iPSCs, and our results show that both genetically edited iPSCs retain their ability to differentiate into NK cells. Preliminary results suggest a synergistic effect between the expression of CAR and the deletion of *KLRC1* in enhancing NK cell anti-tumor activity and we are currently investigating various CARs in combination with our strategy.

Conclusion: There is an urgent need to improve accessibility to cutting-edge treatment, such as cellular immunotherapy. CAR-iNK holds the potential to shift this complex personalized therapeutic option to a universal “off-the-shelf” one. The versatility offered by iPSCs allows for the application of this strategy to other malignancies. Moreover, by studying the loss of function of the targeted negative regulators, we can gain valuable insights and deepen our understanding of the mechanisms underlying NK cell-mediated anti-tumor responses.

Title: Taok3 intrinsically regulates B cell activation and BCR signaling.

Author: Ana Maria Hincapie Restrepo

Affiliation: Michel Tremblay, McGill University

Background information: B cell lymphomas are one of the most common types of blood cancers in young adults. The current standard of care involves combination chemotherapy and CAR T cell therapy. However, many patients experience relapse or do not achieve complete remission. Thus, new treatments are required for the management of this type of cancer, and the only way of achieving this is to better understand the mechanisms driving its development. In normal B cells, activation and proliferation are partly driven by antigen recognition through the B cell receptor (BCR), which then triggers a tightly regulated intracellular signaling cascade. Consequently, mutations or dysregulation of proteins involved in the BCR signaling pathway are often identified in B-cell-derived cancers. Taok3 is a serine threonine kinase that is highly expressed in immune cells, and it is essential in regulating T cell activation and limiting age-related inflammation. Moreover, Taok3 is essential for the development of a subset of B cells known as marginal zone B cells. However, whether Taok3 regulates B cell activation and related lymphomagenesis remains unknown.

Purpose of the study: To dissect the specific role of Taok3 in B cell development and activation, with a focus on its possible role in BCR signaling and the malignant transformation of B cells.

Methods: Immunophenotyping of Taok3 full-body and Taok3 B-cell-specific knockout mice has been carried out using flow-cytometry-based assays. B cells were isolated from the spleen and lymph nodes of the Taok3 knockout mice and wild-type controls. Activation-induced proliferation was analyzed using flow cytometry and the B cell receptor signaling cascade was analyzed using western blotting. Serum levels of immunoglobulins were measured using ELISA.

Results: We have observed that Taok3 full-body knockout mice present signs of spontaneous B cell activation, such as the formation of germinal centers and increased serum levels of immunoglobulins. Moreover, we have noted the reduction of B cell populations that are often affected in the presence of strong BCR signaling. To determine whether the phenotype observed is intrinsic to B cells, we have also

characterized Taok3 B-cell-specific knockout mice. Taok3 depletion in B cells leads to a developmental block consistent with enhanced BCR signaling. Additionally, the phosphorylation of proteins involved in early BCR signaling was reduced, suggesting that Taok3 knockout B cells are irresponsive to stimulation through the BCR, likely due to sustained stimulation *in vivo.* Overall, analyses of these mice have demonstrated that Taok3 plays an intrinsic role in the regulation of BCR signaling.

Conclusion: We have demonstrated that Taok3 regulates BCR signaling in an intrinsic manner. Although the specific substrates of this kinase in this signaling cascade remain to be elucidated, our work brings Taok3 forward as a novel regulator of B cell activation. Based on this, we propose this kinase as a potential target for the treatment of diseases related to aberrant B cell activation due to enhanced and sustained BCR signaling, such as B cell lymphomas and specific autoimmune diseases.

Title: Genome-wide CRISPR Screen in B-ALL Unveiling Genes Modulating Natural Killer (NK) Cell Potency Against NK-Resistant Tumor Models

Author: Louise Rethacker

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

Keywords: leukemia; natural killer; cytotoxicity; immunotherapy; resistance

Background information: B-cell acute lymphoblastic leukemia (B-ALL) is the most prevalent cancer in children. Despite the efficacy of chemotherapy, concerns persist regarding its long-term side effects. Presently, research projects aim at developing targeted and less harmful therapies for B-ALL. Natural killer (NK) cells, innate lymphocytes, exhibit cytotoxicity towards stressed-self and/or modified-self cells, including cancer cells. Remarkably, NK cells display potent anti-leukemic activity, and their infiltration into the bone marrow correlates with favorable outcomes in B-ALL patients. Nonetheless, resistance mechanisms and NK cell exhaustion can compromise NK cell function in certain patients.

Purpose of the study: To advance novel therapies or enhance current ones, comprehending the intricate dynamics of NK cell response and its efficacy in targeting B-ALL cells, along with identifying the underlying genes and pathways implicated in this process, is imperative.

Methods: In order to identify pivotal genes governing sensitivity or resistance to NK cell cytotoxicity, we conducted a whole-genome CRISPR screen utilizing three distinct B-ALL cell lines (697, REH and NALM6) exhibiting varying degrees of susceptibility to NK cell lysis. Employing the Yusa CRISPR library, we induced mutations leading to the knockout (KO) of 18,010 genes within B-ALL cells. Then, those cells were cultured with primary activated NK cells, derived from the NK-cell Activation and Expansion System (NKAES), until we reached more than 80% of target cell death. Depending on the target cell line, we did one or two rounds of 24h of co-culture at a ratio 4:1.

Results: Employing the MAGeCK Robust Rank Analysis on surviving cells, we identified the death receptor pathway as the primary mechanism implicated in the NK cell mediated lysis of B-ALL cells. Specifically, sgRNAs targeting FADD, CASP8, BID, FAS, TNFRSF10A and TNFRSF10B were found to be enriched in resistant tumor cells. To validate this finding, we generated cell lines expressing

a dominant-negative FADD protein and confirmed that loss of FADD signaling reduced NK cell-mediated killing of these three B-ALL cell lines. Moreover, as anticipated, analysis of depleted sgRNA in surviving cells demonstrated that loss of HLA-E, HLA-C and B2M increase their sensitivity to NK cell killing. Finally, while some pathways are common across the three cell lines, certain genes are unique to individual cell lines, highlighting the diversity in pathways triggering NK cell killing of B-ALL cell lines.

Conclusion: In summary, our preliminary findings suggest that enhancing the death receptor pathway or implementing blockade of NKG2A and/or KIR receptors may enhance the efficacy of NK cell-based immunotherapy in resistant B-ALL. Currently, we are extending our investigation to explore additional killing pathways in these B-ALL cell lines. Through this screening methodology, our goal is to advance the development of novel NK-based immunotherapies not only for B-ALL but also for various other cancer types.

Title: Genetics of 6-MP related Toxicities in Patients with Acute Lymphoblastic Leukemia

Author: Zarina Sabirova

Affiliation: Maja Krajinovic, Department of Pharmacology and Physiology, Faculty of Medicine, Université de Montréal and CHU Ste – Justine Research Center.

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

Background information: Over the past six decades, the prognosis of childhood lymphoblastic leukemia (ALL) has significantly improved. However, treatment related adverse drug reactions (ADRs) remain a significant threat to the well-being of many patients. Severe myelosuppression, commonly associated with 6-mercaptopurine (6-MP) can lead to life-threatening infections or frequent treatment interruptions. High levels of 6-thioguanine nucleotide (6-TGN) contribute to neutropenia, while accumulation of 6 – methylmercaptopurine (6-MMP) can contribute to hepatotoxicity. Genetic variations can influence a child's susceptibility to ADRs by modulating the effects of drugs used in ALL treatment. Hence, our study aims to identify genetic variants that play a role in influencing the response to 6-MP treatment.

Purpose of the study: 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: Our 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 clinical and pharmacologic outcome measured in ~300 ALL patients treated at CHU Sainte Justine (CHUSJ) with Dana Farber Cancer Institute (DFCI) protocols 2005-00, 2011-01, and 2016-001. 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 included hematological toxicity, such as high-grade neutropenia and hepatotoxicity.

Results: Study findings indicate that genetic variations within the *XO* gene have an impact on the response to 6-MP treatment during the consolidation II and maintenance phases of therapy. Patients with at least one copy of the mutant allele in the rs1884725 SNP experienced a reduced frequency of neutropenia and received higher total doses of 6-MP compared to those with the wild type allele. Additionally, the variant allele in the rs6710015 SNP correlated with both lower levels of 6-TGN and higher 6-MMP metabolites, as well as the development of hepatotoxicity. Moreover, a novel variant, rs73189762, within the *NUDT15* gene had significant associations with the neutropenia and the 6-MP dose intensity. Carriers of the variant allele received lower doses of 6-MP and demonstrated increased susceptibility to neutropenia during treatment.

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.

Titre : Caractérisation des fonctions moléculaires du facteur de transcription NR4A3 lors de la réponse des lymphocytes T CD8.

Auteur : Eric Gyres Sonounameto

Affiliation : Nathalie Labrecque, Département de médecine, Institut de recherches clinique de Montréal

Mots clés : Cellules T CD8+, Réponse immunitaire, Leucémie, Thérapie, NR4A3

Informations générales : Une réponse anti-leucémique optimale requiert des lymphocytes T (LT) CD8+ efficaces contre la tumeur. Toutefois, en cas d'infection chronique ou de cancer, les LT s'épuisent, perdant en fonctionnalité. La population de LT épuisés (LTex) est hétérogène et caractérisée par une expression accrue de récepteurs inhibiteurs (PD-1, Tim-3, Lag-3). Les LTex se divisent en trois sous-populations : les progéniteurs (LTex prog), les effecteurs-like (Eff-like) et les terminaux. Les LTex prog maintiennent la réponse immunitaire face à une infection chronique ou un cancer et répondent au blocage des points de contrôle. Nous avons identifié le facteur de transcription (FT) NR4A3 comme étant important dans la différenciation des LT CD8+ en effecteurs et en mémoires. Nous émettons l'hypothèse que NR4A3 pourrait réguler également la différenciation et la fonction des LTex lors d'infections chroniques ou de cancers.

Objectif de l'étude : Déterminer l'impact de la déficience en NR4A3 sur la différenciation et la fonction des LT CD8+ lors d'une infection chronique LCMV-Clone13 (Lmphocytic Choriomeningitis Virus-Clone 13).

Méthodes : Les LT CD8 P14, possédant un récepteur de cellules T (TCR) transgénique spécifique pour le peptide (gp33–41) du LCMV, restreint par H-2Db, ont été transférés dans des receveuses B6.SJL. Le jour suivant le transfert, les souris ont été infectées avec du LCMV-Clone 13. La réponse à l'infection a été suivie a été suivie à jour 8 et à jour 30, en récupérant la rate et en faisant des marquages des d'épuisements (PD1, Tim-3, Lag-3) et des populations de LT épuisés progéniteurs (SLAMF6+, Tim3-) ; effecteurs (CX3CR1+, CD101-) et terminaux (CX3CR1-, CD101+). Pour évaluer les fonctions effectrices des LT CD8+ P14, elles sont restimulées ex-vivo avec le peptide gp-33 pendant 5_h suivi d'un marquage des cytokines effectrices (IFN-g, IL2, TNF-a) et molécule effectrice (Granzyme B).

Résultats : Nos résultats montrent que les LTex déficients en NR4A3 à jour 8 en réponse à l'infection chronique se différencient plus en LTex prog de manière significative par rapport aux LTex non déficientes. Cependant, cette différence significative est perdue à jour 30. Nos résultats suggèrent que NR4A3 régule la très tôt la différenciation des LTex en LTex prog. Cependant les mécanismes moléculaires par lesquels NR4A3 contrôle la différenciation des LTex restent incompris.

Conclusion : Ces résultats soulignent le rôle crucial du FT NR4A3 dans la régulation de la génération des LT progéniteurs durant les infections chroniques. A l'aide du single cell-RNAseq et du single cell ATAC-Seq nous évaluerons les différences transcriptomiques et l'accessibilité à la chromatine en absence de NR4A3 afin de comprendre comment NR4A3 régule la différenciation des LTex.

Title: Treating B-Cell Acute Lymphoblastic Leukemia With CAR-Engineered HSCs

Author: Clara Soulard

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

Keywords: B cells acute lymphoblastic leukemia, Hematopoietic stem cells, Cell-type specific promoters, Chimeric antigen receptor, Humanized mice

Background information: Acute Lymphoblastic Leukemia (ALL) is the most common pediatric cancer and the leading cause of death related to cancer in children. Over the past decade the adoptive transfer of T cells modified to express a chimeric antigen receptor (CAR) has emerged as a promising therapeutic approach. CARs are artificial receptors designed to recognize specific surface antigens on target cells, triggering an activation cascade within the genetically engineered immune cells, ultimately leading to the destruction of the targeted cells. Despite frequent good efficacy of CAR therapies, some patients do not respond or will relapse in part due to poor persistence, T-cell exhaustion and/or leukemic cell evasion.

Purpose of the study: We aim to develop an alternative therapeutic approach in which CAR transduction is made in hematopoietic stem cells (HSCs), instead of T cells, allowing a continuous and progressive replenishment of CAR modified cells.

Methods: The constitutive expression of a CAR in the whole hematopoietic compartment highly increases the risk of disrupting cell differentiation process and/or activation of a proto-oncogene. To enhance the safety of CAR-HSCs, we decided to restrict CAR expression only to T and NK cells. Using a bioinformatic approach, we designed a synthetic T/NK cell specific promoter. We transduced HSCs with a lentiviral vector coding for the Green Fluorescent Protein (GFP) reporter gene controlled by the T/NK synthetic promoter. We validated the specificity of the synthetic promoter by both *in vitro* differentiation of GFP engineered HSCs, and injection in humanized mice. Next, we transduced HSCs with a CAR targeting the B cell surface antigen, CD22, either driven by a constitutive promoter or by the T/NK promoter. We evaluated the impact of CAR expression on T cell development. Finally, we humanized mice using CAR-CD22 engineered HSCs under the control of the T/NK specific promoter and challenged them with a human B-ALL cell line.

Results: Our results showed that we were able to generate new synthetic promoters with a lineage specificity. We demonstrated in humanized mice that HSCs engineered with a CAR-CD22 controlled by the human elongation factor-1 (EF1)-a constitutive promoter failed to differentiate into mature T cells. However, by substituting the EF1-a promoter for our synthetic T/NK promoter in the CAR cassette, we were able to generate mature CAR-T cells. Mice engrafted with HSCs transduced with the CAR-CD22 under the control of the T/NK specific promoter showed normal immune reconstitution without B cell depletion. Nevertheless, when they were challenged with B-ALL cells line, mice injected with CAR engineered HSCs showed a delay in leukemia progression and a significant increase in overall survival.

Conclusion: By combining our synthetic promoter technology and the use of HSCs for CAR transduction, our approach could overcome past hurdles and increase the efficacy of CAR immunotherapies while reducing their toxicity.

Title: Enhancement of the graft-versus leukemia effect through the modulation of Neuropilin-1 expression in the context of allogeneic hematopoietic stem cell transplant

Author: Ali Smaani

Affiliation: Jean-Sébastien Delisle, Maisonneuve-Rosemont Hospital Research Center, Université de Montréal

Keywords: Leukemia – Graft – Immunotherapy – Neuropilin-1

Background information:
Neuropilin-1 (NRP1) is a membrane co-receptor highly prevalent in tumors that is associated with the immunosuppression mechanisms leading to tumor escape. NRP-1 is expressed in a multitude of immune cell populations (myeloid and lymphoid). It has been demonstrated that NRP-1 is expressed on populations with immunoinhibitory properties such as regulatory T lymphocytes (Treg) and macrophage subtypes and that its deletion attenuates the suppressive effects. We also find NRP-1 on activated effector CD8 T cells and its expression can lead to a defect in their effector functions. NRP-1 does not render CD8 completely dysfunctional, but rather appears to promote an exhaustion phenotype. The exact mechanisms of modulation of CD8 activity by NRP-1, however, remain to be elucidated. An increase in the expression of NRP-1 has also been observed in leukemic cells and in the bone marrow of patients with AML and this expression has been associated with a poor prognosis. The most common treatment for AML is chemotherapy, which may be accompanied by a hematopoietic stem cell transplant (SCT). The most common SCT is the allogeneic transplant which consists of using stem cells from a partially histo-incompatible donor. Allogeneic transplantation comes with high risks of GVHD but also the potential to induce GVL and protect the patient from relapse.

Purpose of the study: By first elucidating the function of NRP1 in various immune cells and tumor models, our project aims to modulate NRP1 expression in the context allo-HSCT in a way that will enhance the GVL effect while limiting the risk and severity of GVHD.

Methods: We assess the expression of NRP1 at different stages of T cell activation in vitro and assess the impact of NRP-1 overexpression in CD8 using a retroviral vector to better observe its inhibitory effects. We use

flow cytometry to characterize T cells and mice models to define the role of NRP-1 in tumor and transplant immunology.

Results: Our preliminary data shows that NRP1 is induced only upon activation of CD8 T cells and its expression is directly proportional to the intensity of the activating signal. NRP1 does not seem to have any effect on the effector functions of CD8 T cells even when incubated with known NRP1 ligands. The expression of NRP1 correlates with the expression of the exhaustion markers PD-1 and 2B4. NRP1 is induced in the tumor microenvironment and is highly expressed in tumor infiltrating CD8 T cells. Our retroviral vector has shown great transduction efficiency as evidenced by a 3-fold increase in surface expression of NRP1. When adoptively transferred into tumor-bearing mice, T cells transduced with the NRP1 vector show a better infiltration compared to an empty vector control.

Conclusion: NRP1 expression is highly correlated with activation, intra-tumoral infiltration and expression of exhaustion markers in CD8 T cells. We anticipate that NRP-1 expression will also correlate with tissue and tumor infiltration in the context of transplantation to treat leukemia/lymphoma. Future studies will determine whether NRP-1 gain or loss of function impacts GVHD and GVL.

Title: Atomic-level observation of RAS GTPase activity in live cells using NMR spectroscopy

Author: Regina Strakhova

Affiliation: Matthew Smith, Molecular Biology Department, IRIC

Keywords: RAS GTPases, oncogenic mutants, signaling, NMR spectroscopy, structural biology

Background information: RAS small GTPases orchestrate multiple signaling pathways. Binding to GTP allows canonical GTPases to adopt an active conformation and interact with various downstream effectors; thereby determining signalling output (proliferation/survival, gene transcription, programmed death, etc). RAS mutations are found in almost 30% of all human cancers, and two out of three RAS isoforms, NRAS and KRAS, are frequently mutated in Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML) and Juvenile Myelomonocytic Leukemia (JMML).

Purpose of the study: To date, all biophysical data of RAS activity comes from *in vitro* assays. This is a major limitation considering the complexity of RAS-effector signaling pathways in living cells. Thus, we require innovative approaches to study these proteins in their native environment.

Methods: Real-time monitoring of protein conformation using NMR provides detailed, atomic-level insights of enzymatic activity and protein-protein interactions. In-cell NMR spectroscopy (IC-NMR) has the potential to move these analyses into living cells, whereby we monitor the activation and biochemical modifications of RAS and its downstream partners in real time. This is achieved by transducing uniformly (^{15}N) or selectivelly (^{13}C-Isoleucine) isotopically labelled protein purified from *E. coli* into living mammalian cells through electroporation.

Results: We optimized the sample preparation for different cell lines (THP-1, Nalm-6, HEK-293T, HeLa) where protein transduction was verified by microscopy, Western blot analysis and/or NMR spectroscopy. Our current results show that IC-NMR can discern active and inactive states of GTPases, as transduced active mutants exchanged their GDP to GTP in cells post-electroporation. Moreover, we can detect and quantify RAS interactions with small inhibitory molecules (Sotorasib and Adagrasib), binding partners (BRAF-RBD) and visualize multiple GTPases at the same time.

Conclusion: IC-NMR is a powerful technique that will allow us to elucidate how RAS GTPases cycle between active and inactive states, how oncogenic mutations perturb cycling and the mechanisms that dictate signaling output in cells. Detailed biophysical characterization of these key proteins will help us to directly target RAS and ultimately improve outcomes for cancer patients.

Title: Développement, optimisation et validation d'un protocole d'imagerie par résonance magnétique Cardiaque à l'effort chez les patients et les survivants de la leucémie pédiatrique

Author: Egidie Uwase

Co-authors & Affiliation:
Delphine Périé, Maxence Abasq, Maxime Caru; Polytechnique Montréal et Daniel Curnier, Universite de Montréal.

Keywords: Cardiotoxicity; early detection; heart failure; mechanical properties, stress cardiac MRI.

Background information:
Despite various attempts to reduce cardiotoxicity late effects in pediatric leukemia survivors, the risk of developing long-term cardiac disease in this population remains high. Magnetic resonance imaging (MRI) is an effective tool for the advanced characterization of myocardial tissue. To improve the effectiveness of this technique, Exercise Cardiovascular Magnetic Resonance (Ex-CMR) has been developed and allows to reveal cardiac deformities not apparent at rest. However, this technique is not routinely used, due to different limitations including lack of MRI-compatible exercises and the difficulty of acquiring images during exercise. Assessing cardiac tissue mechanical properties from an Ex-CMR technique may improve early detection of cardiotoxicity-induced heart abnormalities in pediatric leukemia survivors.

Purpose of the study: This study aimed to characterize cardiac function and mechanical performance in ALL survivors. We hypothesized that changes in cardiac mechanical properties can reveal subtle pathological deformations induced by cardiotoxicity.

Methods. A total of 63 (ALL) survivors who were classified into three groups: (i.e. standard risk (SR) and high risk with and without cardio-protective agent dexrazoxane (HR and HR+DEX)) underwent both CMR acquisitions on a 3T MRI system and exercise testing with a cycle ergometer. Mechanical parameters including Left Ventricle Stiffness (LVS), LV Contractility (LVC), Cardiac Work Efficiency (CWE), and Ventricular Arterial Coupling (VAC) were computed by the CircAdapt model.

Results. LVS strongly correlated with MRI relaxation time T1, (R^2=94.8 %; p=0.008), and LVC strongly correlated with ejection fraction EF (R^2=93.7 %; p=0.043), in the SR group. CWE was impaired in all groups during rest (< 95 %), thus, demonstrating the impact of doxorubicin-induced cardiotoxicity. During stress, CWE increased in the SR and HR+DEX groups by (5.2 % and 8.0 %, respectively), whereas it increased only by 1.4 % in the HR group. VAC improvement during stress was more limited in the HR group compared to other groups.

Conclusion. Cardiac mechanical properties allow the detection of cardiac abnormalities induced by doxorubicin-related cardiotoxicity treatments. Ex-CMR revealed impaired cardiovascular performance in ALL survivors. Assessment of cardiac mechanical performance using a new stress imaging protocol adapted to the exercise capacity of ALL survivors will allow an effective characterization of cardiotoxicity-induced late adverse. Early detection may improve ALL survivors' follow-up and clinical decision-making.

Title: Characterization of Mast Progenitors in Primary t(8;21) AML

Author: Rui Zhang

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

Keywords: single-cell RNA sequencing, acute myeloid leukemia, mast progenitor, heterogeneity, trajectory analysis

Background information: AML is a highly heterogeneous disease that is stratified into favorable, intermediate, and adverse group. Single-cell genomic techniques have emerged as powerful approaches to characterize different tumors at the resolution of individual cells, even allowing the characterization of rare cell populations. Our group have profiled, using scRNAseq, over 100 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 a novel association between the abundance of mast progenitors and t(8;21) AML subgroup. The recurrence of mast progenitors overabundance across patients with t(8;21) AML suggests that this cell population reflects remarkable properties of this subgroup.

Purpose of the study:
We hypothesize that mast progenitor is an important component, and they may play a significant role in leukemogenesis.

Methods: To identify a gene signature that was specific for mast progenitors in AML samples, I applied unsupervised clustering analysis using Seurat package in R to the scRNAseq data of 10 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 the cell origin of mast progenitors in AML, we probed the fusion transcripts expressed by the cells from our scRNAseq data of t(8;21) samples using a modified km approach. To further explore the heterogeneity of mast progenitors in AML, I applied trajectory analysis to elucidate how these mast progenitors are differentiated from the leukemic stem cells. I also compared the transcriptomic difference for mast progenitor differentiation between AML patients and the healthy individuals.

Results: We identified FCER1A, MS4A2 and IL1RL1 as mast progenitor surface markers across most of the AML samples. We were able to isolate mast progenitors by FACS using these surface markers. We detected the fusion transcripts expressed by the leukemic blasts as well as by mast progenitors in t(8;21) samples. Three trajectories were identified by trajectory analysis including myeloid progenitor, neutrophil progenitor, and mast progenitor. Along the mast progenitor trajectory in t(8;21) AML, stem cell markers are suppressed at the beginning and mast progenitor markers are activated later. We found that IL5RA is a mast progenitor trajectory-specific marker in t(8;21) AML. We also validated that this protein has increased its expression on the cell surface along the mast progenitor differentiation.

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. Their differentiation trajectory between AML patients and healthy individuals is quite similar. However, several aberrant gene expressions have been observed along the mast progenitor differentiation trajectory in t(8;21) AML.