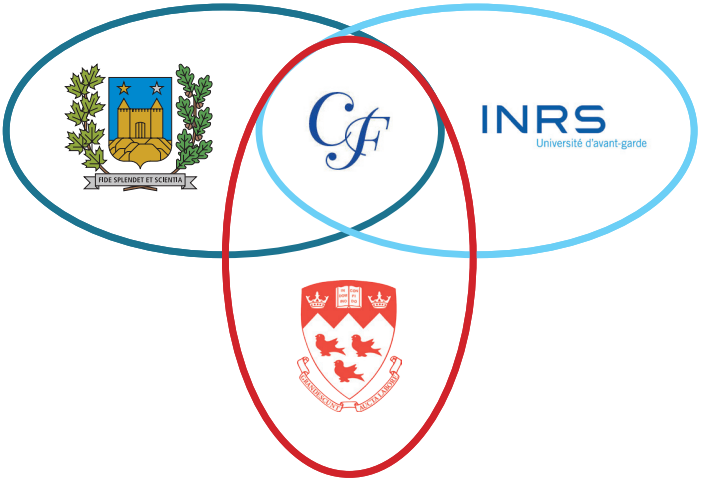
Cole Foundation
«Research Celebration Day»
May 8, 2015



Journée
« Célébrons la recherche »
de la Fondation Cole
8 mai 2015

The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD programmes to promote research inpre-leukemia, leukemia, and other leukemia-related diseases in children andyoung adults, as well as the development of clinical care for patients affected by these diseases. With the announcement of 2015 Fellows, the Fellowship programme has supported more than 129 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 $7 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 child care 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 child care for young patients afflicted with leukemia and leukemia-related diseases.

The Cole Foundation
Barry Cole – President
John Moran – Secretary/Treasurer
David Laidley – Board Member
Anne Lewis – Board Member
Bruce McNiven – Board Member
Dr. Guy Rouleau – Board Member
Dr. Pierre Boyle – Board Member
Dr. Pierre Chartrand – Board Member
Dr. Gerry Batist – Board Member
Gabrielle Cole – Board Member
Viviane Cole – Board Member
Evan Lewis – Board Member
Dr. Sheila Horn Bisaillon – Advisor

La Fondation Cole

La Fondation Cole soutient la recherche sur le syndrome myélodysplasique, 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 post-doctoraux et des étudiants diplômés inscrits à des programmes de doctorat. Avec l'annonce des boursiers 2015, le programme a appuyé plus de 129 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 sept millions de dollars 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 santé s'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.

Program

9 :30 - 10 :00 AM	Round Table (new fellows) *M-425, pavillon Roger-Gaudry*
10 :00 - 11 :00 AM	Poster Session Part 1
11 :00 - 12 :00 PM	Poster Session Part 2
12 :00 - 12 :45 PM	Lunch/Dîner
1 :00 - 2 :00 PM	Lecture *Hall d'honneur, pavillon Roger-Gaudry*
Welcome	*Dre Hélène Boisjoly, Doyenne de la Faculté de médecine, Université de Montréal*
New Research - New Realities Presentations	*Dr. Jean-Sébastien Delisle* *Hôpital Maisonneuve-Rosemont* *« The human T cell as an anti-leukemia weapon ; from T-cell biology to clinical trials »*
	Dr. Hugo Wurtele *Hôpital Maisonneuve-Rosemont* *« Understanding the mechanism of action of histone deacetylase inhibitors to improve leukemia treatments »*
	Dr. Heather Melichar *Hôpital Maisonneuve-Rosemont* *« T cell suppression by a novel co-stimulatory molecule interaction with implications in the treatment of GVHD »*
2 :00 - 3 :15 PM *Introduction*	*Barry Cole*
Keynote Speaker	*Dr Lawrence Cooper* *Section Chief of Cell Therapy, Children's Cancer Hospital, MD Anderson Cancer Center, Houston, Texas*
	« Advancing Genetically Modified T Cells from the Bench to the Bedside »
3 :15 - 3 :30 PM	New fellows and announcement of prizes
3 :30 PM	Reception

The 2015 - 2017 Cole Foundation Fellows

Les boursiers de recherche de la Fondation Cole 2015 – 2017

Université de Montréal

Karine Gauvin Bourdages, PhD program
Supervisor : Mike Tyers, IRIC, Medecine
Project title : Genome-wide CRISPR gene knockout collection in human cells
Description : The Fbw7 tumor suppressor gene is mutated in 31% of T-cell acute lymphoblastic leukemia. In order to help identify new therapeutic targets for cancers bearing mutations in Fbw7, CRISPR knockout technology will allow us to survey the entire genome for the occurrence of co-lethal interactions with Fbw7

Sophie Cardin, Post PhD program
Supervisor : Sonia Cellot, CHU Sainte-Justine, Pediatrics
Project title : Investigating the rôle of histone déméthylases in pédiatrie leukemia
Description : Histone methylation patterns regulate cellular transcriptional programs and contribute to cell fate decisions in hematopoiesis and leukemia.
Characterizing the role of Jumonji demethylases in human hematopoietic stem cells and acute myeloid leukemia will contribute to our understanding of the disease and potentially identify novel drug targets

Jun Chen, Post PhD program
Supervisor : André Veillette, IRCM, Molecular Oncology
Project title : SLAMF7 is the pro-phagocytic receptor on macrophages for elimination of leukemia and lymphoma
Description : We are working on identifying the pro-phagocytic receptor on macrophages and elucidating its mechanism of action. We hope through using this knowledge to develop new treatments to enhance tumor elimination of childhood leukemia and lymphoma, and decrease the use of non-specific treatment regimens

Xi-Lin Chen, Post PhD program
Supervisor : Katherine Borden, IRIC, Pathology and Cell Biology
Project title : eIF4E3 inhibits oncogenic eIF4E mRNA export activity by modulation its subcellular trafficking
Description : We will investigate the mechanisms by which eIF4E3 inhibits oncogenic eIF4E subcellular trafficking and mRNA export activity. The outcome of this project may help to develop novel therapeutic strategies in acute myeloid leukemia

Kristelle Desfossés Baron, PhD program
Supervisor : Hugo Wurtele, Hôpital Maisonneuve-Rosemont, Medecine
Project title : L'acide valproïque, un possible traitement pour la leucémie pédiatrique, module la réparation et la réplication de l'ADN
Description : Clinic trials are underway to test the therapeutic efficacy of molecules called deacetylase inhibitors in the management of leukemia, a malignancy that is often highly refractory to treatment. Our research aims to better understand the molecular mechanisms underlying the anti-cancer effects of deacetylase inhibitors to permit rational optimization of pediatric leukemia treatment

Ludivine Litzler, PhD program
Supervisor : Javier Di Noia, IRCM, Genetic Diversity
Project title : Oncogenic interaction of PRMT5 and AID in B cell acute lymphoblastic leukemia
Description : Our lab studies mutagenic mechanisms that allow B lymphocytes to produce competent antibodies after an infection. My project aims at understanding the normal and oncogenic role of an enzyme involved in those mechanisms and that is deregulated in B cell neoplasms. It represents a potential target for B cell lymphomas

Adam-Nicolas Pelletier, Post PhD program
Supervisor : Brian Wilhelm, IRIC, Medecine
Project title : Characterization of RET in MLL-AF9 acute myeloid leukemia
Description : We are currently characterizing an receptor tyrosine kinase (RTK) found to be differentially expressed in MLL-AF9 leukemias. Knockdown of this novel candidate gene induces apoptosis of leukemic cells, through undetermined mechanisms. Thus, a better understanding of this the biological function of this RTK could allow development of novel therapeutic approaches

Swati Ganesh Shetty, PhD program
Supervisor : Guy Sauvageau, IRIC, Medecine
Project title : A genetic approach to identify molecular pathways relevant to novel anti-leukemia drugs.
Description : Engineering of a KBM-7 cells library, which will contain clones harboring random homozygous chromosomal deletions. This will serve as a tool to improve our capability to identify novel molecular pathways relevant to the sensitivity and resistance of drugs that we have identified to inhibit cell growth in several-patient derived AML specimens

Camille Simon, PhD program
Supervisor : Guy Sauvageau, IRIC, Medecine
Project title : The Polycomb Repressive Complex 2 (PRC2) : a mechanistic investigation of its role in normal and leukemia stem cells
Description : The Polycomb Group genes regulate the activity of normal hemopoietic and leukemia stem/progenitor cells. Indeed, recurrent mutations and miss-regulation affecting these genes are often observed in various types of blood cancers. This project aims to understand the mechanisms mediating their functions and to evaluate the potential of Polycomb genes as therapeutic targets

Elisa Tomellini, Post PhD program
Supervisor : Guy Sauvageau, IRIC, Medecine
Project title : Optimization of NK cell precursor production to improve anti-leukemia activity and reduce disease relapse
Description : Today many patients are deprived of access to hematopoietic stem cells (HSC) transplant procedures because the HSC number requirement cannot be met, leading to delayed immune reconstitution and graft failure. The aim of this research project is to use chemogenomic approaches to uncover cellular pathways essential to HSC proliferation and differentiation

Aurélie Tormo, Post PhD program
Supervisor : Moutih Rafei, Université de Montréal, Pharmacologie
Project title : Interleukin-21-based therapy for T-cell reconstitution following bone marrow transplantation
Description : Pre-conditioning treatments such as radio- and chemotherapies prior to bone marrow transplantation (BMT) lead to substantial thymic damages impairing T-cell development. Consequently, patients undergoing BMT do not re-establish functional immunity. Thus, our major aim is to investigate the capacity of interleukin-21 at accelerating/enhancing the re-acquisition of immune functions following BMT

Diogo Veiga, Post PhD program
Supervisor : Trang Hoang, IRIC, Pharmacologie
Project title : A novel drug discovery approach for targeted therapy in childhoot T-cell acute lymphoblastic leukemia
Description : A novel drug discovery approach for targeted therapy in childhood T-cell acute lymphoblastic leukemia (T-ALL). Current T-ALL chemotherapy is efficient in destroying cancer cells, but also has undesired side- effects due the lack of specificity. We will implement a novel drug discovery pipeline to specifically inhibit events that are critical for T-ALL initiation and progression, i.e. self-renewal and pre-TCR signalling

McGill University

Marina Bury, Post PhD program
Supervisor : Volker Blank, Lady Davis Institute, Medecine
Project title : Role of the NFE2L3 transcription factor in cellular stress response and lymphomagenesis
Description : Our Nfe2l3 null mice exhibit higher morbidity, mortality and developed an increased number of lymphomas upon exposure to the carcinogen benzo[a]pyrene. My studies of the gene regulatory networks involved in lymphomagenesis will provide new insights helping in the design of novel treatment tools for patients with hematological malignancies

Michael Förster, Post PhD program
Supervisor : Anastasiya Nijnik, McGill Life Sciences, Physiology
Project title : Mysm1 as a potential drug target for p53 activation in hematological malignancies
Description : With this work, we aim to investigate the feasibility of targeting the p53 pathway in hematological malignancy through a novel regulator, the deubiquitinase Mysm1 that is specifically expressed in the hematopoietic system thereby defining whether Mysm1-inhibition is a viable strategy to induce p53-activation preferentially within hematopoietic tissues of leukemic patients

Cole Foundation Poster Session
Session d'affiches de la Fondation Cole

Presenters / Liste des exposants

Aissiou, Mohamed
Assaker, Gloria
Barbour, Haithem
Bolt, Alicia
Celton, Magalie
Dupéré-Richer, Daphne
Gerby, Bastien
Grapton, Damien
Guégan, Jean-Philippe
Guo, Qianyu
Izreig, Said
Itoua Maiga, Rayelle
Kachaner, David
Krakow, Elizabeth
Lavallée, Vincent-Philippe
Lemay, Valérie
Maiga, Arhamatoulaye
Mancini, Johanna
Montpas, Nicolas
Motorina, Alena
Rouette, Alexandre
Sen Nkwe Dibondo, Nadine
Simon, Camille
Thiollier, Clarisse
Wang, Peng
Zahreddine, Hiba

Poster List / Liste des projets exposés

No.	Name	Title
PO1	Aissiou, Mohamed Doc	Early detection of doxorubicin-induced cardiotoxicity using multi-parametric mri : proposed model for childhood leukemia
PO2	Assaker, Gloria Doc	Identification of novel regulators of the Notch ligands Delta-Like1 and 4 as potential therapeutic targets for T-cell Acute Lymphoblastic Leukemia (T-ALL)
PO3	Barbour, Haithem Doc	Epigenetic regulation by the leukemia suppressor TET2
PO4	Bolt, Alicia Post-Doc	Investigating the potential role of tungsten on leukemogenesis
PO5	Celton, Magalie *(absent)*	Model leukemia to study the development and the evolution of AML with the MLL-AF9 translocation
PO6	Dupéré-Richer, Daphne Doc	HDACi resistance in lymphoma cells is associated with increased proteasome inhibitors sensitivity
PO7	Gerby, Bastien Post-Doc	Targeting pre-leukemic stem cells in T-acute lymphoblastic leukemia

PO8	Grapton, Damien *(absent)*	Study of epigenetic changes induced by the variant form of GFI1 (GFI136N) in the development of acute myelogenous leukemia and myelodysplatic syndrome
PO9	Guégan, Jean-Philippe Post-Doc	ERK1/2 regulates the R-ras family proteins
PO10	Guo, Qian-yu Doc	Darinaparsin induces G2/M cell cycle arrest and BRG1 phosphorylation to inhibit antioxidant cytoprotective machinery : A novel anti-leukemic mechanism
PO11	Izreig, Said *(absent)*	MIR-17-92 REPRESSION OF LKB1 IS NECESSARY FOR MYC-DRIVEN METABOLIC REPROGRAMING IN LYMPHOMA
PO12	Itoua Maiga, Rayelle Doc	Structure/Function Analysis of the c-MYC Proto-oncogene mRNA
PO13	Kachaner, David Post-Doc	Interdomain allosteric regulation of Polo kinase by Aurora B and Map205 is required for cytokinesis
PO14	Krakow, Elizabeth Clinician	A Phase I Trial of Alloreactive Cell Therapy without Substantial Permanent Engraftment to Treat Poor-Prognosis Acute Myeloid Leukemia
PO15	Lavallée, Vincent-Philippe Clinician	The transcriptomic landscape and directed chemical interrogation of *MLL*-rearranged acute myeloid leukemias
PO16	Lemay, Valérie Doc	Une aptitude aérobie réduite chez les survivants de leucémie lymphoblastique aigüe : quels sont les facteurs associés?
PO17	Maiga, Arhamatoulaye Post-Doc	Disruption of the 5'untranslated region of an oncogene using the CRISPR/Cas9 system
PO18	Mancini, Johanna Doc	Selective Telomere Shortening in Cancer Cells by a G-quadruplex Ligand

PO19	Montpas, Nicolas Doc	Role of CXCR7 as a negative modulator of leukemia cells in bone marrow niche homing
PO20	Motorina, Alena Doc	Oncogene collaboration in pediatric acute megakaryoblastic leukemia
PO21	Rouette, Alexandre Doc	Le rôle de l'immunoprotéasome dans l'oncogenèse
PO22	Sen Nkwe Dibondo, Nadine Doc	Role of nuclear exclusion of the deubiquitinase USP16 in coordinating DNA damage signaling
PO23	Simon, Camille Doc	*Ezh1* and *Ezh2* are essential regulators of normal and leukemic hematopoietic stem cells
PO24	Thiollier, Clarisse Post-Doc	A new marker for Acute Myeloid Leukemia with poor prognosis
PO25	Wang, Peng Post-Doc	Deciphering the Role of PP2A in Translation Control in Leukemia
PO26	Zahreddine, Hiba Doc	eIF4E coordinately upregulates Hyaluronic acid synthesis and production of its receptor CD44 to increase aggressiveness of AML cells

PO1

Title: Early detection of doxorubicin-induced cardiotoxicity using multiparametric mri: proposed model for childhood leukemia

Author: Mohamed Aissiou

Affiliation: Delphine Périé-Curnier, Mechanical Engineering Department, École Polytechnique de Montréal, Montreal, Canada.

Keywords: doxorubicin cardiotoxicity, cine MRI, T1 and T2 imaging, childhood leukemia.

Background information: Doxorubicin chemotherapy is effective and widely used to treat acute lymphoblastic leukemia. However, its effectiveness is hampered by a wide spectrum of dose-dependent cardiotoxicity including both morphological and functional changes affecting the myocardium. Currently, very few techniques are available for estimating myocardial damage. The use of muscle fibers orientation (e.g., diffusion tensor imaging DT-MRI) or 3D imaging techniques (e.g., cine DENSE MRI) are promising alternatives, however, their clinical application is limited due to the acquisition time and their estimation errors.

Purpose of the study: The aim of this study was to propose an imaging-based method to early detect changes in myocardial tissue and deformation using T1 and T2 imaging along with clinical cine MRI. The hypothesis is that doxorubicin chemotherapy induces direct damage to myocardial fibers architecture which can be detected from either changes in T1 and T2 relaxation times within the myocardium or altered deformation.

Methods: We retrospectively included 26 patients (mean age: 25.037.7 years) which had previously undergone a doxorubicin-based chemotherapy treatment for childhood leukemia, following the Boston protocol. The mean doxorubicin doses administered to patients was 241397 mg/m^2 while mean dexrazoxane doses was 27493463 mg/m^2. Patients were divided into two groups: high risk (HR, n=18) and standard risk (SR, n=8) patients, based on the cumulative doxorubicin dose received during treatment, age at the beginning of the treatment and other clinical factors. T1 (MOLLI) pre- and 10-min post-gadolinium and T2 data were acquired for each patient during breath-holds and ECG-gating, using a Siemens 3T MR system. In parallel, whole-heart cine images were acquired in 2-chamber, 4-chamber and short axis views with a pixel resolution of 1.4x1.4x8.0mm. Myocardial contours were extracted using an interactive curve-based segmentation, from which, myocardial skeletons were calculated and used to divide the myocardium into

segments, where T1 and T2 relaxation times mean were estimated. In parallel, myocardial displacement were estimated along with clinical indices including thickening and ejection fractions. To compare our results, we included 9 healthy volunteers that have undergone the same acquisition parameters for T1 pre- and post-gadolinium enhancement.

Results: Pre-gadolinium T1 and T2 relaxation times were similar between SR and HR groups. Post-gadolinium T1 was higher in SR group in 11 out of 19 segments, predominantly at mid-ventricular and apical levels. Linear regressions between mean T1 and T2 and several clinical indices showed no relationships (R^2<0.2) for patients' age, gender, weight, received doxorubicin dose or time between end of treatment and MRI acquisition. Mean T1 pre-/post-gadolinium and T2 (base: 1313/597/45ms, middle: 1283/598/45ms, apex: 1297/599/46ms) were respectively higher for T1 pre-gadolinium, higher for T1 post-gadolinium and similar for T2 relaxation times as compared with reported values (base: 1157/427/44ms, middle: 1159/411/45ms, apex: 1181/400/47ms). Left ventricular systolic and diastolic indices in all patients were 5037% and 49321%, while for healthy volunteers they were 5339% and 48317%. Some changes in ventricular areas, skeleton lengths and wall thickness were noted mainly in terms of phase duration within the cardiac cycle, however these changes were local, while no important dysfunctions were seen.

Conclusion: Local myocardial changes of T1 pre- and post-gadolinium were found between HR and SR patients. However, T2 relaxation times failed to detect any differences between HR and SR patients. The use of T1 relaxation times, especially post-gadolinium T1 as early biomarker should be further investigated. On the other hand, deformation patterns analysis showed no significant differences between HR and SR patients, suggesting that the use of dexrazoxane as a cardioprotective agent may have curbed doxorubicin-related cardiotoxicities.

PO2

Title: Identification of novel regulators of the Notch ligands Delta-Like1 and 4 as potential therapeutic targets for T-cell Acute Lymphoblastic Leukemia (T-ALL)

Author: Gloria Assaker

Affiliation: Dr Gregory Emery, Vesicular Trafficking and Cell Signaling laboratory, Institute for Research in Immunology and Cancer (IRIC)- Université de Montréal

Keywords: Notch, Delta-like, screen, T-ALL.

Background information: The signaling receptor Notch plays a key role in stem cell self-renewal, cell proliferation, and differentiation. Consequently, it is involved in many diseases and cancers, including childhood Acute Lymphoblastic Leukemia (ALL), which represents the most common pediatric malignancy. The Notch receptor responds to transmembrane ligands of the DSL family (Delta/Serrate/Lag-2) and many cancers involving Notch are ligand-dependent. For instance, activating mutations in *NOTCH1* have been identified in over 55% of T-ALL, with 40% of these mutations resulting in ligand hypersensitivity or ligand-independent Notch activation. Strikingly, the molecular mechanisms that regulate ligand activation in the signal-sending compartment are yet to be characterized.

Purpose of the study: The aim of this project is to identify regulators of the Delta-like ligands activity that subsequently modulate Notch signaling and its oncogenic activity in T-ALL.

Methods: Using a co-culture assay with a luciferase Notch reporter, we performed the first genome-wide RNAi screen aiming specifically at identifying regulators of Notch ligands in the signal-sending cell. We next developed functional secondary screens where we demonstrated that the identified Delta-like1 and 4 regulators are important for Notch-mediated events, such as normal T-cell differentiation, and survival of pre-leukemic stem cells (pre-LSCs) isolated from a mouse model of T-ALL. Finally, we showed that some hits are also required for LSC activity *in vivo* following transplantation. Interestingly, using RNA-seq data from a recent study (*St-Pierre et al., 2013*), we found the majority of our top hits to be well expressed in the thymic compartment where the Notch ligands are highly enriched, which strongly argues for the functional relevance of these regulators in the modulation of ligand-dependent Notch signaling *in vivo*.

Results: Taken together, our screen and the validation experiments allowed us to identify new classes of Notch pathway regulators such as protease inhibitors, transcription factors and other genes of previously uncharacterized function**.** Hierarchical clustering of these hits based on our luciferase and functional screening data highlighted genes that are known to share similar or related functions and that cluster together in our assays; including genes involved in the pathogenesis of T-ALL. Accordingly, our transplantation experiments in mice demonstrate that pre-LSCs require ligand activation of Notch to induce leukemia, and validate our hits as potential molecular targets for the treatment of T-ALL.

Conclusion and Perspectives: Overall, this study led to the identification of novel regulators of Delta-like ligands that could serve as potential therapeutic targets in Notch cancers, as exemplified by the T-ALL model. We are currently performing molecular characterization of these hits in order to elucidate their mechanism of action on the ligands, which will be critical to develop pharmacological inhibitors to counteract their action and impede oncogenic Notch signaling.

PO3

Title: Epigenetic regulation by the leukemia suppressor TET2

Authors: Haithem Barbour[1], Haider H. Dar[1], Salima Daou[1], Jessica Gagnon[1], Nadine Sen[1], Ian Hammond-Martel[1] and El Bachir Affar[1]

Affiliation: [1] Centre de Recherche Hôpital Maisonneuve-Rosemont, Université de Montréal, Canada.

Keywords: Leukemia, TET2, 5hmC, OGT, GlcNAc

Background information: TET2 is a major tumor suppressor and a highly mutated gene in multiple types of hematological malignancies including acute-myeloid leukemia (AML). TET2 converts the methylated cytosine (5mC) repression mark to hydroxyl-methyl-cytosine (5hmC) which facilitates the activation of gene transcription. Several TET2 loss-of-function mutations have been documented in leukemia, however the dynamics behind TET2 catalytic activity and coordination of interacting partners are far from being fully understood. One of TET2 major interacting partners is the O-linked GlcNAc transferase (OGT), which adds a GlcNAc group to serine and threonine residues of TET2.

Purpose of the study: Our aim is to establish the mechanism by which TET2 and OGT interact with each other, and with their own protein complex partners in normal and leukemic cells. We also want to unveil the impact of defects in these interactions in leukemic transformation.

Methods: To fulfill our goals, we used multiple methods including protein Tandem-Affinity Purification (TAP) to purify the TET2-OGT complexes which were subjected to mass-spectrometry analysis to identify the interacting partners. We also used western blot and dot-blot techniques to evaluate both TET2 stability and catalytic activity depending on its interaction with OGT.

Results: Our results show that OGT interacts with and glycosylates TET2 on specific residues. We also found that OGT affects TET2 catalytic activity towards 5mC. To determine TET2 and OGT expression levels in several cancer types, we analyzed more than 800 high throughput RNA-seq datasets from the TCGA database. We also used the same datasets to conduct gene expression correlation studies of TET2 to predict leukemia-specific target genes. Our RNA-seq analysis suggest that the expression of both of TET2 and OGT are significantly amplified specifically in leukemic cells (AML) and we have also identified potential TET2 target genes whose expression strongly correlate with the expression of TET2. Our results suggest that TET2 could be regulated through two major pathways: first the interaction with OGT which directly modulates it activity and second the interaction with other proteins which modulate it

recruitment to target genes. The amplification of TET2 mRNA levels is highly correlated with the expression of other epigenetic regulators specifically in leukemia which could explain the disordered epigenetic landscape in this pathology.

Conclusion: Our study should help in a better stratification of leukemia cells according to gene expression signatures coordinated by TET2 and OGT.

PO4

Title: Investigating the potential role of tungsten on leukemogenesis.

Author: Alicia Bolt

Affiliation: Dr. Koren Mann, Lady Davis Institute, Department of Oncology, McGill University

Keywords: Tungsten, Bone Marrow, Hematopoiesis, Cell Differentiation, and Leukemogenesis

Background information: Tungsten is a naturally occurring metal that has several desirable properties including strength and flexibility. Historically tungsten was thought to be "inert" with low toxicity, which has led to its incorporation into a variety of household goods and medical devices. Increased use of tungsten has resulted in increased contamination of air and ground water sources, especially near active mines and industrial sites containing tungsten. This has raised public concern over the lack of toxicological data investigating the potential human health risks. Recently, high levels of tungsten were identified in the drinking water near three pediatric leukemia clusters in the United States, yet the contribution of tungsten to the development of leukemia has not been defined.

Purpose of the study: Tungsten rapidly accumulates in the bone of C57BL/6 mice in a concentration-dependent manner, making the bone and bone marrow microenvironments potential sites of tungsten toxicity. The purpose of this project is to define what effects tungsten has on B cell lymphopoiesis in order to identify potential "hits" required for leukemogenesis.

Results and Conclusions: Wild-type C57BL/6 mice exposed to tungsten in their drinking water over a 16-week exposure had altered B-cell development and increased DNA damage within the bone marrow. Tungsten-exposed mice had an accumulation of pre-B cells at 16 weeks, the same B-cell stage commonly found in pediatric leukemia. We have developed a 7-color flow cytometry panel in order to FACS-sort this pre-B cell fraction from the bone marrow of control and tungsten-exposed mice. Gene expression of the C/C' pre-B cell fraction after a 4 week exposure to 15 ppm (μg/mL) tungsten was evaluated by RNA-Seq. We observed that tungsten down-regulated the expression of multiple genes known to be master regulators of B-cell commitment and development including Pax5, Erb1, Notch1, Runx1, and Foxp1. Interestingly, tungsten suppressed both the IL-7 and preBCR receptor signaling pathways critical for B-cell differentiation at the C/C' pre-B cell stage and suggest that tungsten may be halting B-cell differentiation. In addition, we are using an inducible TEL-AML1 mouse model of leukemia to evaluate whether tungsten can provide one of the 2-hits required for leukemogenesis. Based on the initial set of mice analyzed, 9 out of

22 tungsten-exposed, TEL-AML1 mice developed some type of disease pathology with 23 % developing splenomegaly and 18 % developing intestinal enteritis. While splenomegaly and intestinal enteritis are not definitive markers of leukemia, these are promising results that suggest that tungsten can induce immune pathologies in this model, which we are currently characterizing. In summary, tungsten targets hematopoiesis by altering B-cell differentiation and inducing DNA damage. Based on the RNA-Seq analysis, tungsten alters B-cell development by down-regulating multiple signaling pathways crucial for pre-B cell differentiation. In addition, based on the TEL-AML1 animal model, we have promising results to suggest that tungsten is altering immune responses, potentially providing one of the 2-hits required for leukemogenesis.

PO5

Title: Model leukemia to study the development and the evolution of AML with the MLL-AF9 translocation.

Author: Magalie Celton

Affiliation: Dr. Brian Wilhelm, Laboratory for high-throughput genomics, IRIC, Université de Montréal

Keywords: MLL-rearranged AML, NGS, DNA methylation, epigenetics

Background information: Mixed Lineage Leukemia (*MLL*) translocations are common in pediatric leukemias. One of the most frequent *MLL* fusion partner genes in pediatric AML is the gene *MLLT3* (encoding the AF9 protein). Next generation DNA sequencing (NGS) has significantly contributed to identifying new therapeutic targets, however the molecular mechanisms underlying oncogenesis in leukemias associated with rearrangement of the *MLL* gene remains unclear.

Purpose of the study: This project focuses on investigating the molecular mechanisms involved in the development of MLL-AF9 (MA9) AML by the identification of novel candidate genes specific to MA9 AML. We also focus on understanding the evolution of gene expression patterns during leukemogenesis by correlating changes in DNA methylation to gene expression changes.

Methods: Because DNA sequencing from several pediatric MA9 AML patients did not identify novel common mutations, we have generated engineered MA9-AML leukemias in collaboration with Dr. Fred Barabé. This novel model system uses primitive human hematopoietic cells, expressing a human MLL fusion gene, to create AMLs with features that recapitulate human leukemia. These can then be compared to patient AMLs to identify consistent transcriptional changes. Because MA9 translocations cause changes in DNA methylation, we have been using our model AML system to study the stepwise, genome-wide, changes in DNA methylation patterns. This is done using an in-solution hybrid capture procedure (Agilent SureSelect) combined with a bisulfite treatment to survey DNA methylation in CpG islands and promoters.

Results: We have generated 22 model leukemias from 4 single donors, performing RNA sequencing at each step, and comparing these to data from our pediatric patients. This analysis has revealed 39 candidate genes expressed in MA9 AML samples but silenced in other subtypes of AML and in non-leukemic cells. To understand the mechanism for their AML-specific expression, we have generated bisulfite-converted DNA libraries from model AMLs (initial $CD34^+$ cells,

CD34^{+} cells transduced with MA9 and the final AMLs) and three MA9-AML patients. Our analysis has revealed that, despite the immediate expression changes after the MA9 fusion gene is added, there is a dramatic increase (>10x) in DNA methylation changes when the transformed cells are xenotransplanted into mice. This observation, which is consistent with our methylation data from patients, suggests that the *in vivo* niche of AML cells has a profound impact on the transcriptional regulation of the cells during their leukemic transformation.

Conclusion: Our single donor model AML system provides an experimental system to remove the confounding effects arising from a mixed genetic background. It has allowed us to identify AML specific transcriptional changes and has revealed consistent changes in the location and dynamics of DNA methylation. These data will ultimately help to highlight novel therapeutic targets which are critical in the process of leukemogenesis.

PO6

Title: HDACi resistance in lymphoma cells is associated with increased proteasome inhibitors sensitivity

Authors: Daphné Dupéré-Richer[1], Mena Kinal[1], Filippa Pettersson[1], Audrey Edmond[1], Jessica N. Nichol[1], Cynthia Guilbert[1], Dany Plourde[1], Kathleen Klein Oros[11], Torsten H. Nielsen[1], Jonathan D. Licht[4], Nathalie A. Johnson[1], Sarit Assouline[1], Leandro Cerchietti[2], Wilson H. Miller Jr.[1*], Koren K. Mann[1*]

Background information: Epigenetic modifying enzymes are commonly mutated in diffuse large B cell lymphoma (DLBCL) suggesting that the regulation of histone protein acetylation is an important factor in DLBCL pathogenesis and can be potential targets for therapy. These mutations persist at relapse from standard therapy (5, 6), and thus provide an opportunity for second line therapy. Although histone deacetylase inhibitors (HDACi), as a single agent showed modest objective response rates in DLBCL ranging from 5.5% (7) to 25% (8), there is evidence for greater efficacy when used as a chemo- sensitizer (9). Using epigenetic drugs along side conventional therapy has been shown to significantly increase cancer cell death *in vitro* and *in vivo*. The purpose of this study was to understanding HDACi-induced changes in unresponsive DLBCL in order to potentially aid in the development of combination therapies.

Methods: We developed cell lines resistant to the histone deacetylase inhibitor (HDACi) vorinostat, in order to better define the molecular mechanisms of action of HDACi in lymphoma cells. Using whole genome expression analysis, we found that the most significantly deregulated genes are included in pathways involved in the processing of proteins i.e. lysosome, protein catabolic process, ER membrane and Golgi vesicle transport. Consistent with this, we find that HDACi resistant cells have increased ER stress and proteasomal degradation. Importantly, the resistant cell line has a marked increase in proteasome inhibitor susceptibility *in vitro* and in mice xenografts. Moreover, using transcriptional signatures found in our resistant lymphoma cell line model, we show that tumors from DLBCL patients treated, but unresponsive to HDACi therapy, undergo similar changes that reflect the presence of proteotoxic stress,

Conclusion: DLBCL cells and patient tumors treated with HDACi have undergone genetic re-programming to accommodate an increase in proteotoxic stress which supports sensitization to proteasome inhibitor as a viable therapeutic strategy for HDACi use in DLBCL in the clinic.

PO7

Title: Targeting pre-leukemic stem cells in T-acute lymphoblastic leukemia

Author: Bastien Gerby

Affiliation: Dr. Trang Hoang, Hematopoiesis and Leukemia Research research unit from IRIC.

Keywords: SCL, LMO, self-renewal, pre-leukemic stem cell, targeted therapy.

Background information: Current chemotherapy of pediatric T cell acute lymphoblastic leukemia (T-ALL) efficiently reduces the tumor mass and induces long-term remission. Nonetheless, pre-leukemic stem cells (pre-LSCs) that were not active during treatment are spared and can eventually evolve to malignancy causing relapse. Moreover, current treatment is associated with undesirable consequences including reproductive, obesity and muscoluskeletal problems and remains ineffective in adolescent and adult T-ALL. Using transgenic mouse models expressing the SCL and LMO1 oncogenic transcription factors in the thymus, we recently showed that these oncogenes reprogram normal pro-T cells into aberrantly self-renewing pre-LSCs[1].

Purpose of the study: Based on the evidence that pre-LSCs in AML are resistant to current induction therapy[2,3], we proposed a strategy to identify drugs that inhibit the cell viability of pre-LSCs maintained by co-culture with stromal cells expressing the NOTCH1 ligand DL4 to mimick the thymic microenvironement.

Methods: We developed a robust high throughput (HTS) phenotypic screen of a library of 3581 chemical compounds. This screening confirmed chemotherapeutic agents commonly used in the treatment of T-ALL and revealed new compounds.

Results: Surprisingly, for relatively quiescent cells, we identified microtubule-targeting drugs (MTDs) as the most robust and consistently active family of inhibitors, suggesting new and unexpected function of MTDs. Indeed, we report that the MTD UM0119979 abrogates pre-LSCs viability and self-renewal activity by inhibiting SCL function. Moreover, the UM0119979 exhibits a potent anti-leukemic effect on primary human T-ALL blasts *in vitro* and *in vivo* without affecting normal HSCs functions.

Conclusion: Together, our findings demonstrate the feasibility of targeting pre-LSCs and identify a new drug for consideration in T-ALL treatment.

1. Gerby B et al. PLoS Genet. 2014;10(12):e1004768
2. Corces-Zimmerman MR et al. Proc Natl Acad Sci. 2014;111:2548-2553.
3. Ding L et al. Nature. 2012;481:506-510.

P08

Title: Study of epigenetic changes induced by the variant form of GFI1 (GFI136N) in the development of acute myelogenous leukemia and myelodysplatic syndrome

Author: Damien Grapton[1], Lars Michel[2], Lacramioara Botezatu[2], Judith Hönes[2], Anne Helness[1], Charles Vadnais[1], Cyrus Khandanpour[2] and Tarik Möröy[1]

Affiliation: 1IRCM, Canada; 2UC Essen, Germany

Keywords: Acute Myeloid leukemia, myelodysplastic syndrome, GFI1, transcription factors, epigenetic

Background information: Acute myeloid leukemia (AML) and myelo-dysplastic syndrome (MDS) are hematological malignancies. AML is characterized by an increase in the proliferation of myeloid progenitor cells and their impaired differentiation in the bone marrow (BM). MDS is often considered a pre-AML stage where progenitor cells exhibit impaired differentiation and eventually progress into AML. We have observed that loss of the transcription factor Gfi1, leads to an accumulation of myeloid cells in mice that is reminiscent of MDS. Our laboratory discovered a subgroup of patients with a single nucleotide polymorphism (SNP) in the GFI1 gene, which causes a serine at the position 36 to be substituted by an asparagine (GFI136N). Patients with the GFI136N variant have a higher risk of developing AML.

Purpose of the study: We hypothesize that the variant form of Gfi1 could have a specific role in AML development and maintenance but also in MDS-to-AML transition.

Methods: Our team generated “knock-in” mice carrying either the human wild type GFI1 (Gfi136S/36S) or the human variant GFI136N (Gfi136N/36N) at the locus of the murine GFI1. To further study the role of Gfi136N in AML development and maintenance, we use a mouse strain that expresses the human oncofusion protein CBFB-MYH11 (inv(16)) frequently found in AML patients. To study the influence of GFI136N in MDS development, we also crossed the Gfi136S/36S and Gfi136N/36N with transgenic mice expressing the fusion protein NUP98-HOXD13 (NHD13) in hematopoietic cells. Finally, I will use chromatin immune precipitation and high throughput sequencing (ChIP-Seq) to compare the state of methylation and acetylation of histone H3 on the entire genome of cells expressing GFI136S or GFI136N. We will also perform transcriptomic analysis by RNA-Sequencing (RNA-Seq)

Results: Recent data from our team suggest that GFI136N predisposes patients to MDS and increases the risk of developing fatal AML from a pre-existing MDS by 3-fold. Mice carrying GFI136N and expressing CBFB-MYH11 showed a significantly accelerated onset of AML compared to mice carrying GFI136S (p=0.004). In addition NHD13 transgenic mice carrying Gfi136N developed AML with a higher incidence (55% vs 25%) and shortened latency (410 vs 360). Using ChipSeq and RNASeq, we found that mice expressing Gfi136N display globally higher levels of dimethylated H3K4 and acetylated H3K9 which are activation marks with overrepresentation of genes involving pathways of cytokine signaling, hematopoietic lineage development and AML genesis. We have evidence that the GFI136N variant is defective in mediating histone modifications and we hypothesize that this deficiency causesthe rapid progression of GFI136N positive MDS patients to AML.

Conclusion: Our data suggest that Gfi136N is a novel predictive marker for AML development among MDS and AML patients. We are currently working to determine the genome-wide histone methylation and -acetylation levels in leukemic cells from knockin mice models that carry the human GFI136S and GFI136N alleles and patients that have progressed from MDS to AML. Furthermore, we plan to determine the effect of histone methyltransferase and -acetyltransferase inhibitors in GFI136N or GFI136S knockin mice with AML. Notes

P09

Title: ERK1/2 regulates the R-ras family proteins

Author: GUÉGAN Jean-Philippe

Affiliation: Dr Meloche Sylvain, IRIC, Université de Montréal

Keywords: TC21, R-ras1, MAPK, signaling pathway, leukemia

Background information: The small GTPase TC21, also known as R-ras2, belongs to the R-Ras family of protein and shares numerous activators and effectors with the classical Ras proteins. Therefore and similarly to Ras, deregulation of TC21 activity has been correlated with the onset and/or progression of different malignancies including various hematopoietic tumors. TC21 is necessary for the survival and the proliferation of B- and T-cells and it exhibits an increased expression in myelodysplastic syndrome, in some lymphoid/myeloid chronic leukemias and in T lymphomas. Nevertheless, the regulation of the GTPase TC21 remains largely unknown. Recently, we discovered in a large scale phosphoproteomic analysis that TC21 could be phosphorylated by the kinases ERK1/2. Given the hyperactive status of the MAPK pathway in many hematopoietic tumors, the phosphorylation of TC21 could have a significant impact on its activity and thus on leukemogenesis.

Purpose of the study: In this study, we aim to validate the regulation of TC21 by ERK1/2 and to understand the impacts of this regulation on the protein TC21 and its pro-oncogenic capabilities.

Methods - Results: By using GST- pulldown and co-immunoprecipitations assays, we first showed that TC21 physically interacts with the kinases ERK1/2, leading to TC21 phosphorylation. Indeed, we validated both *in vitro* (kinase assay) and *in vivo* (Phos–tag technology) that the protein TC21 is phosphorylated on its serine 186. This residue is located in a full consensus site of phosphorylation by ERK and is evolutionary conserved. We also showed that an equivalent phosphorylation, also performed by ERK1/2, could be found on TC21 isoform R-Ras1. By performing xenograft experiments, we then demonstrated that the phosphorylation of TC21 or R-Ras1 by ERK could enhance the oncogenic potency of both small GTPases. In fact, overexpression of TC21 or R-Ras1 is sufficient to transform normal fibroblasts and we showed that the expression of the phospho-mimetic mutant of these GTPases accelerates the growth of xenografts in nude mice. Finally, we showed by Raf-RBD pulldown assays that the phosphorylation by ERK1/2 directly stimulates the intrinsic activity of R-Ras1 but not TC21. For the latter protein, the aim of this regulation is still under investigation but our preliminary results showed that the phosphorylation did not affect the basal localization of TC21 or its stability.

Conclusion: The phosphorylation of the R-ras proteins by ERK1/2 could therefore represent a major mechanism of regulation of these small GTPases and their signaling. These results strengthened the interest of the MAPK pathway targeting in leukemias, in which this signaling pathway has been shown to be hyperactivated and a source of resistance to current therapies.

P010

Title: Darinaparsin induces G2/M cell cycle arrest and BRG1 phospho-rylation to inhibit antioxidant cytoprotective machinery: A novel anti-leukemic mechanism

Author: Qian-yu Guo

Affiliation: Wilson H. Miller Jr., Segal Cancer Centre, Jewish General Hospital, Division of Experimental Medicine, McGill University

Keywords: Darinaparsin, G2/M arrest, BRG1, leukemia

Background: Therapeutic advances have improved acute myeloid leukemia (AML) remission rates, however, approximately one-half of AML patients inevitably relapse. Arsenic trioxide (ATO) has been used successfully to treat acute promyelocytic leukemia (APL), a subtype of AML, but has limited promise in other AML subtypes due to its systemic toxicities at therapeutic dosage. Darinaparsin (DAR; ZIO-101, Sdimethylarsino-glutathione), a novel organic arsenical, has shown superior anti-leukemic activity to ATO, but the mechanism(s) behind its enhanced cytotoxicity remain unelucidated. Our previous studies suggest that ATO induces heme oxygenase (HO-1) expression via recruitment of brahma-related gene 1 (BRG1), an ATPase of the SWI/SNF chromatin remodeling complex, to the HO-1 gene (HMOX1) promoter, thus partially protecting the cells against ATO-induced oxidative stress. DAR fails to induce HO-1 expression, as it leads to the phosphorylation and inactivation of BRG1, as well as G2/M cell cycle arrest.

Purpose of the Study: Unknown signaling networks lead to DAR-induced BRG1 phosphorylation. We hypothesize that mitogen-activated protein kinase kinase (MAPKK)/extracellular signal-regulated kinase (ERK) pathway mediates this phosphorylation, as a previous study has suggested that ERK promotes BRG1 phosphorylation in G2/M arrest.

Methods: HeLa, K562, NB4 and RKO cells were obtained from American Type Culture Collection (ATCC) and cultured as recommended. Protein expression was examined by western blotting. Cell cycle profiles were characterized with propidium iodide staining and fluorescence-activated cell sorting (FACS). mRNA levels were compared by realtime PCR.

Results: First, DAR induces G2/M cell cycle arrest, BRG1 hyper-phosphorylation and MAPKK1 activation with similar temporal kinetics in HeLa cells. Second, DAR-induced BRG1 hyper-phosphorylation, and consequential inhibition, of HO-1 expression can be reversed by U0126, a MAPKK-1/2 inhibitor. Finally, the G2/M arrest and BRG1 hyperphosphorylation in response to DAR is observed in several hematological and solid malignant cell line models, suggesting that DAR's cytotoxic action extends beyond AML.

Conclusion: Our results highlight the DAR-induced MAPKK1-dependent posttranslational modification of BRG1 as a novel mediator for enhancing the therapeutic range of arsenicals.

P011

Title: MIR-17-92 REPRESSION OF LKB1 IS NECESSARY FOR MYC-DRIVEN METABOLIC REPROGRAMING IN LYMPHOMA

Author: Said Izreig

Affiliation: Dr. Russell Jones lab, Goodman Cancer Research Center, Department of Physiology, McGill University

Keywords: Lymphoma, miRNA, metabolism, phenformin

Background information: The proto-oncogenic transcription factor Myc is commonly implicated in human cancers, and is able to orchestrate a pro-cancer metabolic shift when deregulated. While Myc itself has proven to be difficult to target directly, downstream effectors of Myc engaged in metabolic reprograming in cancer may be more amenable to therapy. The miRNA cluster miR-17-92 is a transcriptional target of Myc, and is potentially involved in metabolic regulation in cancer.

Purpose of the study: To explore the mechanism by which Myc regulates cellular metabolism in lymphoma. To develop rational therapies that take advantage of metabolic sensitivities of lymphoma cells.

Methods: To investigate the molecular basis of Myc-driven metabolic reprograming, a Myc-driven lymphoma model with conditional deletion of miR-17-92 was employed. Mass isotopomer labelling coupled to gas chromatography – mass spectrometry was employed to investigate flux of labelled metabolites through multiple metabolite pools. The Seahorse Extracellular Flux Analyzer and NOVA bioprofiler were also employed for real-time measurements of cellular metabolic activity.

Results: Deletion of miR-17-92 caused significant decreases in glycolytic and oxidative metabolism. The miR-17 component of the cluster was found to negatively regulate the master metabolic regulator and tumour suppressor LKB1. Knocking down expression of LKB1 in cells lacking miR-17-92 rescued metabolic activity and tumourigenicity *in vivo*. Reduced LKB1 expression has been shown to sensitize cells to phenformin, a drug used previously for diabetes. *In vivo* administration of phenformin to tumour bearing mice dramatically reduced morbidity, and in some cases cured mice of lymphoma.

Conclusions: The powerful metabolic reprogramming engendered by Myc is due, in part, to miR-17-92. Therapeutic intervention using phenformin is rationalized and demonstrated to be effective as a single agent in lymphoma bearing mice. By investigating downstream effectors of Myc activity, miR-17-92 and LKB1 signaling have been identified as being essential in Myc-driven metabolism in lymphoma. Given this understanding, metabolic therapies aimed at these molecular targets may provide new avenues for treatment of Myc-driven lymphomas.

P012

Title: Structure/Function Analysis of the c-MYC Proto-oncogene mRNA

Author: Rayelle, Itoua Maiga

Affiliation: Jerry Pelletier, Department of Biochemistry, McGill University

Keywords: Myc, 5'UTR, CRISPR/Cas9, genome editing, translation

Background: In the past decades, several elements of the cap-dependent translation apparatus have been associated with tumorigenesis and/or chemoresistance. Briefly, during cap-dependent initiation, eukaryotic initiation factor 4F (eIF4F) complex is recruited to the 5' cap of the mRNA. This complex consists of a cap binding subunit (eIF4E), a larger scaffolding protein (eIF4G) and an RNA helicase (eIF4A). This step is rate-limiting for translation and a check point for linking PI3K/mTOR and Ras-MAPK signalling activity to protein synthesis. Structural barriers in mRNA 5' untranslated regions (5'UTR) increase their dependency on eIF4F activity. Among transcripts with long, structured 5' UTRs is Myc, an extensively studied transcription factor, that is known to be an eIF4F-responsive mRNA. Translocations, some of which truncate the Myc 5' UTR, lead to its overexpression and are known drivers in cancers such as Burkitt's lymphoma and childhood medulloblastoma.

Purpose of the study: The aim of this study is to define the MYC 5' UTR structure/function relationships.

Methods: We use a powerful mouse model, the *Eu-Myc* model, in which MYC expression becomes deregulated during B cell differentiation, to study oncogene cooperation in lymphomagenesis. For example, previous experiments performed in the *Eu-Myc* stem cells have shown that p53 suppression cooperates with elevated MYC levels to induce lymphomagenesis. This suggests a screen that can be used to identify elements regulating MYC expression. To define elements in the MYC 5' UTR that are involved in suppression of its expression, we have targeted this region in wild-type stem cells using the CRISPR/Cas9 system. Co-suppression of p53 in these cells, followed by re-implantation into wild-type C57BL/6 recipients lead to tumor formation with some of the modified cells.

Results: We are currently focusing on the characterization of the several mice that developed lymphomas. We are determining the mutation burden, consequences on MYC mRNA and protein expression, and cellular phenotype.

Conclusion: Increasing efforts are being made to understand the regulation of transcripts encoding key oncogenic functions required for tumor initiation and tumor cell maintenance. These experiments will define information within the MYC 5' UTR that is necessary to the regulation of its expression.

P013

Title: Interdomain allosteric regulation of Polo kinase by Aurora B and Map205 is required for cytokinesis

Author: David Kachaner

Affiliation: Dr Vincent Archambault, Cell Cycle Regulation research unit, IRIC, Université de Montréal

Keywords: Cell cycle, Mitosis, Cytokinesis, Polo kinase, Leukemia

Background information: Cancer is characterized by excessive cellular proliferation. The Polo kinase is an essential regulator of mitosis and cytokinesis and its inhibition causes mitotic catastrophe and cell death. The Polo kinase (called Plk1 in Humans) is over-expressed in a variety of malignancies including pediatric acute myeloid leukemia (AML) and serves as a negative prognostic marker in specific human cancer types.

Purpose of the study: Drosophila Polo and its human ortholog Polo-like kinase 1 fulfill essential roles during cell division. Members of the Polo-like kinase (Plk) family contain an N-terminal kinase domain (KD) and a C-terminal Polo-Box Domain (PBD) which mediates protein interactions. The purpose of this study consists to investigate how is the Polo kinase regulated during cytokinesis, the last step of cell division.

Methods: We have investigated this question using *Drosophila melanogaster* as a model and its cells in culture. We used both biochemistry (coIP, kinase assays, GST pull-down) and cellular biology (immunofluorescence, live cell imaging) approaches to decipher the regulation of the Polo kinase during cytokinesis.

Results: Our results indicate that phosphorylation of the Polo kinase by Aurora B is required for cytokinesis. This phosphorylation in the activation loop of the KD promotes the dissociation of Polo from the PBD-bound microtubule-associated protein Map205, which acts as an allosteric inhibitor of Polo kinase activity. This mechanism allows the release of active Polo from microtubules of the central spindle and its recruitment to the site of cytokinesis. Failure in Polo phosphorylation results in both early and late cytokinesis defects. Importantly, the antagonistic regulation of Polo by Aurora B and Map205 in cytokinesis reveals that inter-domain allosteric mechanisms can play important roles in controlling the cellular functions of Plks.

Conclusion: Collectively, our results illuminate the molecular mechanisms that regulate the Polo kinase during cell division and may suggest new therapeutic avenues that will disrupt Polo functions in cancer cells.

P014

Title: A Phase I Trial of Alloreactive Cell Therapy without Substantial Permanent Engraftment to Treat Poor-Prognosis Acute Myeloid Leukemia

Author: Elizabeth Krakow**,** Denis-Claude Roy, Julie Bergeron, Silvy Lachance, Jean-Sébastien Delisle

Affiliation: Blood and Marrow Transplant Program, Maisonneuve Rosemont Hospital / Université de Montréal

Keywords: Acute myeloid leukemia, alloreactivity, HLA mismatch, clinical trial

Background: Human leukocyte antigen (HLA) mismatched donor cell infusions outside the context of stem cell transplantation (SCT) may offer the anti-leukemic effects of alloreactivity without substantial risk of graft-*versus*-host disease (GVHD). Haploidentical or higher-order mismatched G-CSF mobilized peripheral blood mononuclear cells (PBMCs) and purified or enriched NK cell products have been used to treat AML in small studies and in one randomized controlled trial, and are being further developed in increasing numbers of trials worldwide. The optimal donor, cell composition, cell dose, and mechanisms of action relevant to transient alloreactive cell therapy are unknown.

Objective: We initiated a phase I trial to test the safety of steady-state mismatched PBMCs to treat chemorefractory or relapsed AML (ATAC-AML-01, ClinicalTrials.gov NCT01793025).

Methods: ATAC-AML-01 is an ongoing prospective dose-escalation trial conducted at our institution enrolling patients with primary refractory or relapsed AML who are not eligible for stem cell transplantation. Key exclusion criteria include poor organ function, prior autologous stem cell transplantation, and prior purine analogue chemotherapy. PBMCs from a haploidentical or higher-order HLA-mismatched donor are harvested by apheresis and infused 24-48 hours after induction (if not in remission) or after consolidation chemotherapy. At least 3 patients are treated at each of 4 dose levels ranging from 1 Ð 10^7 to 2 Ð 10^8 CD3/kg (expanding to 6 patients if 1 experiences dose-limiting toxicity). The primary outcome is the dose at which ≤ 33% of patients experience DLT. Secondary outcomes to be reported descriptively include causes of mortality, incidence of GVHD, duration of cytopenias, complete remissions, and relapse-free and overall survival.

Progress and Outlook: To date, 5 patients have been treated. We have not observed acute infusional toxicity, prolonged aplasia, or GVHD. Of 4 patients evaluable for response, 3 attained CR and 1 did not respond. In one instance, ATAC served as bridge to SCT; that patient is alive in CR 26 months after ATAC. The other patients who attained CR experience gradual relapse after 77 days and 164 days post ATAC, and were then treated with azacitidine. In order to optimize this approach in future trials, biologic correlates of response will be studied, such as the duration of microchimerism and recipient-derived leukemia-specific T cell responses that might arise after bidirectional alloreactivity.

P015

Title: The transcriptomic landscape and directed chemical interrogation of *MLL*-rearranged acute myeloid leukemias

Author: Vincent-Philippe Lavallée

Affiliations: The Leucegene project at IRIC, Université de Montréal. Josée Hébert : Banque de cellules leucémiques du Québec, Hôpital Maisonneuve-Rosemont. Guy Sauvageau : Guy Sauvageau Laboratory, IRIC, Université de Montréal.

Keywords: Acute myeloid leukemia; *MLL*; transcriptomics; *RAS*

Background information: The *Mixed Lineage Leukemia* (*MLL*) gene is recurrently translocated in a subset of pediatric and adult acute leukemias. Translocations can involve various fusion partners, and most subtypes are associated with an adverse clinical outcome. To date, mutations in adult acute myeloid leukemias with *MLL* translocations (hereafter termed *MLL*-t AML) have only been investigated by targeted approaches, and differentially expressed genes explored in microarray studies. We hypothesized that next-generation sequencing (NGS) of a large cohort of *MLL*-t and control AML could provide key insights in this disease.

Purpose of the study: To determine the transcriptomic and mutational landscape of *MLL*-t AML, and to perform a targeted chemical screen based on the recurrent genetic anomalies identified.

Methods: Transcriptomes (n=31) and corresponding exomes (n=29) of primary *MLL*-t AML samples were sequenced and compared to the transcriptomes of 384 control non-*MLL*-t AML, which also included 23 specimens with *MLL* partial tandem duplications (*MLL*-PTD). Analysis of differential gene expression between cohorts was performed using the Wilcoxon rank-sum test and the false discovery rate (FDR) method was applied. All mutations among a set of 55 genes recurrently mutated in hematological malignancies are reported. In addition, any other gene with ≥ 3 variants in the *MLL*-t cohort was investigated in non tumoral DNA in order to identify novel acquired mutations. Targeted chemical screens using MEK and receptor tyrosine kinase (RTK) inhibitors as single drugs and in combinations were performed on primary *MLL*-t samples with and without *RAS*-pathway mutations.

Results: We identified a signature composed of the 140 genes most differentially expressed between *MLL*-t and non-*MLL*-t AML. Five adjacent genes on chromosome band 15q13.1 represented the most significant differentially expressed transcripts. Among those, upregulation of *LOC100289656* was confirmed experimentally and high expression of this transcript in the control cohort allowed the identification of 4 cryptic *MLL* fusions, including a new *MLL-ENAH* fusion. *LOC100289656* was also significantly overexpressed in

MLL-PTD, and represents the first transcriptomic unifying network common to *MLL*-t and *MLL*-PTD. A subset of *MLL*-t specimens (3/31) carried novel mutations in *SPI1* accompanied by inactivation of its transcriptional network. *MLL*-t samples also harbored frequent (14/31) *RAS*-pathway mutations which sensitized leukemias to synthetic lethal interactions between MEK and RTK inhibitors compared to *MLL*-t AML without such mutations.

Conclusion: Our genetic analyses identified the transcriptomic signature of *MLL*-t AML, revealed that *MLL*-t and *MLL*-PTD AML are linked by the overexpression of adjacent genes on chromosome 15, and identified a novel *MLL* fusion transcript and new *SPI1* mutations in this disease. Chemical interrogation of *RAS* mutations revealed an additional angle unforeseeable by transcriptomics only: the presence of synthetic lethal interactions between MEK and RTK inhibitors in *RAS*-mutated *MLL*-t AMLs. Our results thus demonstrate that combining NGS with in vitro targeted chemical screens enabled further characterization of *MLL*-t AML. Such integrative chemo-genomic studies will likely be useful for the comprehensive analysis of other leukemia subtypes and of solid tumors.

P016

Titre: Une aptitude aérobie réduite chez les survivants de leucémie lymphoblastique aigüe: quels sont les facteurs associés?

Auteurs: Valérie Lemay[1-2], Gregor Andelfinger[1], Daniel Sinnett[1], Maja Krajinovic[1], Caroline Laverdière[1], Marie-Eve Mathieu[2], Daniel Curnier [1-2]

Affiliations: [1]CHU Sainte-Justine, Département hémato-oncologie; [2]Département de kinésiologie, Université de Montréal

Mots clés: physiologie de l'exercice, cardiotoxicité, leucémie lymphoblastique aigüe, activité physique

Introduction: Le taux de survie des enfants atteints de leucémie lymphoblastique aigüe (LLA) se chiffre maintenant à près de 85%. Par contre, plus de 2/3 des survivants sont confrontés plusieurs années à la fin de leurs traitements à des problèmes de santé chroniques tel que de l'obésité, des troubles métaboliques, une densité osseuse réduite ou des problèmes cardiaques. Bien que tous les résultats des études ne convergent pas, la majorité suggère que l'aptitude aérobie des survivants LLA est réduite par rapport à la population générale. Une connaissance précise de l'impact physiologique des traitements vs une pratique d'activité physique (AP) insuffisante sur le déconditionnement observé permettrait sans doute de réconcilier les données discordantes de la littérature.

Buts de l'étude: 1) Présenter un portrait de la pratique d'AP des survivants LLA; 2) présenter un portait de leur réponse cardiorespiratoire à l'effort maximal; 3) Identifier les facteurs reliés à la maladie et aux traitements qui sont reliés à la pratique d'AP et l'aptitude physique de ces sujets.

Méthode: 170 survivants LLA (48% hommes, 22,2 3 6.6 ans, 43% risque LLA élevé; 57% risque LLA standard) ont été évalués au CHU Sainte-Justine 14.1 3 5.6 ans après la fin de leurs traitements. Ils ont complété un questionnaire de rappel d'activités physiques et sédentaires portant sur les trois derniers mois et ont complété une épreuve d'effort maximale sur bicyclette ergométrique. Les paramètres cardiorespiratoires à l'effort ont été mesurés à l'aide d'un analyseur de gaz et les paramètres hémodynamiques ont été mesurés avec un ECG 12 dérivations, prises de tension artérielle et cardiographie d'impédance (PhysioFlow).

Résultats: Les participants pratiquent en moyenne 32 3 35 minutes d'AP de loisir par jour et consacrent 223 3 158 minutes quotidiennent aux activités sédentaires (télévision, ordinateur, jeux vidéos et lecture). Leur dépense énergétique quotidienne totale relative est de 122 3 36 kJkg-1. La consommation d'oxygène de pointe moyenne est de 31.5 3 8.3 ml(kgmin)-1 et le pourcentage de déconditionnement (valeur mesurée vs valeur prédite) moyen est

de -14 3 16%, avec plus de 45% des participants présentant un déconditionnement égal ou supérieure à -15%. Les analyses préliminaires effectuées ne suggèrent pas de limitation évidente au niveau ventilatoire lors d'un effort maximal chez les sujets LLA. Néanmoins, les sujets haut risque présentent un % de déconditionnement supérieur et une ventilation maximale inférieure aux sujets risque standard. Les sujets à haut risque pratiquent également moins d'activités physiques de loisir de haute intensité, mais consacrent aussi moins de temps aux activités sédentaires.

Conclusion: Les résultats des analyses préliminaires appuient les études existantes suggérant un déconditionnement chez les sujets LLA comparativement à la population normale. L'impact de la pratique d'activité physique n'est certainement pas un facteur à écarter pour expliquer ce résultat. D'autres analyses plus détaillées, notamment au niveau des paramètres hémodynamiques à l'effort (débit cardiaque, résistance vasculaire périphérique, etc.) permettront de mieux comprendre les possibles limitations physiologiques retrouvées chez cette population. Les résultats obtenus jusqu'à maintenant soulignent l'importance de développer des programmes d'intervention pour promouvoir la pratique d'activité physique de ces survivants LLA et limiter les comorbidités à long terme.

PO17

Title: GPCR as potential therapeutic targets in acute myeloid leukemia.

Authors: Arhama Maiga, Josée Hébert, Guy Sauvageau, Michel Bouvier.

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

Keywords: GPCR, AML, RNA-Seq, therapeutic target.

Background information: The majority of patients with acute myeloid leukemia (AML) will develop significant side effects due to chemotherapy and/or will eventually relapse and succumb to the disease. There is therefore a real need for the identification of new therapeutic targets for the development of more effective and less toxic treatments.

To identify new potential targets we initiated a large-scale transcriptome sequencing of AML cells obtained from a large collection of well characterized AML samples. Because G protein coupled receptors (GPCR) represent highly drugable targets and given their increasingly recognized role in various cancers, we performed a detailed analysis of their expression levels in AML cells.

Aim: To identify GPCR which expression is deregulated in AML when compared to normal CD 34+ positive cells.

Methods: We used RNAseq to evaluate the expression of 772 GPCR in 152 AML samples of the Banque de cellules leucémiques du Québec (BCLQ) and 12 normal CD34 positive cells sorted from cord blood samples provided by Héma-Québec. The cohort of AML includes 5 different cytogenetic groups. The 772 GPCR analyzed are from the IUPHAR database to which we add 370 olfactory, 24 taste and 4 vomeronasal receptors.

Results and conclusion: Our analysis identified 49 GPCR which median expression is altered in AML cells. Among them, 30 were found to be up-regulated whereas 19 had lower expression. Up-regulated GPCR are enriched in chemokine, prostaglandin and purine receptor family members whereas receptors belonging to the adhesion, protease-activated and Frizzled receptor families were highly represented among the down-regulated ones. GPCR expression level analysis, with regard to the karyotypic abnormalities and the gene mutations detected in the AML cells, showed a significant difference of expression for 20 distinct GPCR when compared to AML samples without the genetic abnormality. Of note, FDA approved drugs already exist for some of the deregulated GPCRs opening the possibility of rapidly testing these compounds in cell-based and *in vivo* systems for both mechanistic studies and the long term perspective of possible drug repurposing.

P018

Title: Selective Telomere Shortening in Cancer Cells by a G-quadruplex Ligand

Author: Johanna Mancini

Affiliation: Dr. Chantal Autexier, Division of Experimental Medicine, Lady Davis Institute for Medical Research, McGill University

Keywords: Telomeres, Telomerase, G-quadruplex, Anti-Cancer, Therapy

Background information: Intact chromosome ends (telomeres), critical for cell proliferation, are maintained by shelterin proteins and by either telomerase or a recombination-based alternative lengthening of telomere (ALT) mechanism. Loss of telomere integrity or extensive telomere shortening activates DNA damage checkpoints, leading to cell death. Detected in ~85% of tumor cells, including acute leukemic cells, telomerase is an attractive target for anti-cancer therapy, but poses several challenges due to the lag associated with telomere shortening and due to activation of the ALT mechanism. An alternative approach is to disrupt telomeres directly, altering interactions between telomeres and their binding proteins. G-quadruplex ligands stabilize structures arising from the folding of single-stranded G-rich 3'-telomere ends (G-quadruplexes), which cannot be elongated by telomerase. Stabilization of these structures can mediate rapid anti-proliferative effects with some specificity in cancer cells. G-quadruplex structures have also been identified in the promoter region of critical proto-oncogenes.

Purpose of the study: Our goal is to determine whether G-quadruplex ligand (PIP) can mediate anti-proliferative effects specifically in cancer cells, to identify the mechanisms mediating these anti-proliferative effects, and to evaluate the effect of ligand treatment on proto-oncogenes associated with leukemia subtypes.

Methods: Using a modified telomerase assay, we confirmed the ability of G-quadruplex stabilizing ligands to inhibit telomerase activity *in vitro*. Based on binding affinity, selectivity, and specificity towards quadruplex DNA, as determined by the fluorescence intercalator displacement (FID) assay, PIP and CLIP were selected for further investigation. Ligand concentration causing 50% cytotoxicity (IC50) was determined in telomerase-positive, telomerase-negative ALT, and non-cancerous primary cells using the MTS metabolic cell proliferation assay. Various dilutions of the IC50 value were used in a long-term seeding assay in order to assess the effect of ligand treatment on telomerase positive A549 cells and on primary MRC5 cells. Cells collected at each reseeding event were

subjected to Telomere Restriction Fragment (TRF) Length analysis in order to investigate the effect of ligand treatment on telomere length. Potential mechanisms of action were investigated through Fluorescence-activated cell sorting (FACS) analysis of the cell cycle, detection of Telomere-dysfunction induced foci (TIFs) through the colocalization of DNA damage marker ĐH2AX and a telomere probe, and induction of senescence via Đ-galactosidase staining.

Results: Our results show that G-quadruplex stabilizing ligands PIN, PIP, PII, PIQ, SIP, and CLIP inhibit telomerase activity in vitro. PIP and CLIP IC50 values were determined and shown to have significant growth inhibitory effects on telomerase-positive and telomerase-negative ALT cells. Various dilutions of the IC50 value were used in a long-term seeding assay. A 0.5X and 0.25X IC50 PIP significantly inhibited the seeding capacity of A549 cancer cells, and a 0.25X IC50 PIP did not affect primary MRC-5 cells. Importantly, A549 cells treated with PIP showed a significant decrease in average telomere length, whereas MRC-5 cells did not. A549 and HUH7 cells, but not MRC5 cells, showed a significant G1 arrest with no signs of apoptosis or DNA damage at the telomere, and stained positive for Đ-galactosidase senescence marker. These results suggest that the observed decrease in cell proliferation and the cancer-specific decrease in telomere length are due to cellular senescence and not apoptosis.

Conclusion: Our results demonstrate that the G-quadruplex stabilizing ligand PIP can significantly affect telomerase-positive cell proliferation and cause increased telomere attrition, specifically in cancer cells, at a dilution of 0.5X IC50. Furthermore, our results suggest that the effects observed are unlikely due to apoptosis but rather due to the early-onset of cellular senescence. Our studies validate the development of novel and specific therapeutic ligands targeting telomeric G-quadruplex structures. G-quadruplex stabilization could provide valuable targeted alterative or combination therapy to cancer and Leukemia patients, while reducing unnecessary side effects of indiscriminate conventional therapies.

P019

Title: Role of CXCR7 as a negative modulator of leukemia cells in bone marrow niche homing

Author: Nicolas Montpas[1,2], Kathie Béland[2], François Fontaine[2], Simon Lamothe[1,2], Simon Nicoletti[2], Geneviève St-Onge[1], Nikolaus Heveker[1,2]

Affiliation: Dr Nikolaus Heveker, [1]Department of Biochemistry, University of Montreal, [2]CHU Ste-Justine Research Center.

Keywords: Chemokine, Bone marrow niches, Homing, MRD, Leukemia

Background information: Leukemia cells can migrate towards, and reside in, specific regions of the bone marrow, called "niches". Leukemia cells "homing" to bone marrow niches relates to persistent **m**inimal **r**esidual **d**isease (MRD) that limited therapy success after chemotherapy and eventually lead to relapse. Indeed, the bone marrow microenvironment provides a relative resistance to chemotherapy to the resident leukemia cells. Our understanding of how leukemia cells migrate to the bone marrow niches, and especially how this process is regulated, is still very limited. The chemoattractant CXCL12 and its receptors CXCR4 and CXCR7 have been identified as key players, but the mechanism used to regulate the bone marrow homing activity is still unknown.

Purpose of the study: The goal of our project is to characterize the role of CXCR7 in leukemia homing to bone marrow niches. More precisely, we want to assess:

1- the expression of CXCR7 in primary leukemic cells and
2- the impact of CXCR7 expression on:
- **a)** CXCR4-mediated migration, and
- **b)** the aggressivity of a specific leukemia in a mouse model.

Methods: Our experiments have been performed on REH (ALL cell line) or on bone marrow biopsies from patients diagnosed with ALL (primary cells). The expression of CXCR4 and CXCR7 has been assessed by flow cytometry using fluorochrome-coupled monoclonal antibody. Dose-response migration experiments have been accomplished on ChemotoX® Disposable Chemotaxis System and the migration potency determined by cell count. We evaluated the time-to-leukemia and time-to-death parameters following intravenous injection of REH or primary leukemic cells in humanized NOD/SCIDgc mice model.

Results: Our results show that the chemotaxis potency mediated by CXCL12 is governed by the ratio of CXCR4/CXCR7 expression rather than by the expression of CXCR4 alone. Unlike CXCR4, CXCR7 is not always expressed in primary cells. Also, preliminary results correlating the expression of CXCR7 in primary cells with the time-to-leukemia and time-to-death will be presented.

Conclusions: Taken together, our results suggest that CXCR7 act as a negative migration modulator of CXCR4. The ratio of CXCR4 and CXCR7 expression in leukemia patients may reveal predictive for bone marrow homing and attachment, and thus for the risk of MRD and relapse.

P020

Title: Oncogene collaboration in pediatric acute megakaryoblastic leukemia

Author: Alena Motorina Affiliation: Dr. Frédérick A. Mallette, Maisonneuve-Rosemont Hospital Research Center, Université de Montréal

Keywords: Acute megakaryoblastic leukemia, fusion oncogenes, oncogene collaboration, tumor suppressors

Background information: Pediatric acute megakaryoblastic leukemias (AMKL), a subtype of acute myeloid leukemia, can be subdivided in two molecularly distinct groups: Down Syndrome and non-Down Syndrome related (NDS). Whereas the prognostic for Down Syndrome AMKL patients is relatively good, NDS-AMKL patients face a particularly aggressive cancer for which the 5-year survival is very low. NDS-AMKL manifests gross chromosomal changes leading to the expression of fusion proteins, the most common of which OTT/MAL, CBFA2T3/GLIS2 and NUP98/JARID1A. Although it is clear that oncogenic fusion proteins involved in AMKL contribute to transformation, transgenic mouse models demonstrated that CBFA2T3-GLIS2 is not sufficient to cause leukemia, suggesting that cooperating oncogenes collaborate with CBFA2T3-GLIS2 to promote leukemia.

In response to mutations leading to oncogenic activation, like those involved in leukemia, such as mutated Ras, Flt3 or IDH2, mammals have evolved cellular responses that oppose neoplastic transformation. One of these responses is cell senescence, a stable cell cycle arrest state where cells remain metabolically active but do not proliferate. Previous data has established that the TEL/JAK2 leukemic fusion induces senescence.

Purpose of the study: As senescence is a tumor suppressive mechanism that must be overcome during cancer development, and that leukemic fusions are not sufficient to cause leukemia, we suggest that hematopoietic malignancies display additional modifications in order to bypass oncogene-induced senescence.
The primary cellular response to fusions involved in AMKL remains elusive. We therefore aim to characterize the requirements for leukemogenesis in AMKL by combining oncogenic fusions to potential collaborating oncogenes.

Methods: In order to verify the primary response to AMKL fusions, we transduced constructs of these fusions into primary human IMR90 fibroblasts and verified the establishment of cellular senescence by markers of OIS such as PML bodies, enlarged cell morphology, loss of E2F gene expression and senescence-associated-ß-galactosidase staining.

Results and conclusion: Preliminary results demonstrated that the CBFA2T3/GLIS2 and NUP98/JARID1A leukemic fusions induced senescence, identifying a novel cellular response to leukemic fusions involved in AMKL. These results will be confirmed in murine hematopoietic stem and progenitor cells, before investigating oncogenes that allow for bypass of the senescent phenotype. We intend to then inhibit directly these oncogenes, re-establishing cell senescence, as a new therapeutic avenue for NDS-AMKL patients, which currently have a very poor prognosis (15-35% survival after 5 years).

P021

Title: Le rôle de l'immunoprotéasome dans l'oncogenèse

Author: Alexandre Rouette

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

Keywords: Leukemia, RNA-seq, Proteasome, Immune Proteasome, Ubiquitination

Background information: Cancer cells are characterized by aneuploidy, chromosomal instability and oxidative stress, leading to imperfect protein homeostasis, like aberrant protein expression and folding. Constitutive proteasomes (CP) are responsible for degradation of most cellular proteins, including those damaged, misfolded and in excess. In addition to CP, vertebrates also express Immunoproteasomes (IP), which display differences in proteolytic activity and cleavage preferences. To date, the known biological roles of IP include non-redundant generation of MHC-I peptides and transcriptional regulation.

Purpose of the study: Bortezomib is a proteasome inhibitor currently used for treatment of multiple myeloma. However, no large-scale studies has assessed whether CP and IP are differentially expressed in several normal vs. neoplastic human tissus, and whether two types of proteasomes play non-redundant roles.

Methods: Using RNAseq data from the Cancer Genome Atlas, we evaluated the expression of IP and CP-encoding genes from 12 cancer types, including 8 control tissus. Survival analyses were performed by comparing the survival of two equal groups of patients separated by the summed zscore of IP- or CP-encoding genes. Correlation analyses were performed by Pearson's coefficient. We monitored protein ubiquitination by Western Blot on AML cell lines of M5 origin (THP1) and M3 origin (NB4, HL60).

Results: Using RNAseq data from the Cancer Genome Atlas, we show that CP- and IP-encoding genes are overexpressed in most cancer types compared to normal counterparts. Furthermore, high expression of IP is associated with poor prognosis in Acute Myeloid Leukemia (AML), and is not associated to markers of immune cells. Moreover, clinical subsets of AML can be classified by their expression of IP both by targeted and unsupervised approaches: M5 leukemias, which are associated to poor prognosis, express high levels of IP while M3 expressed low levels. In addition, THP1 cells, but not NB4 or HL60, show rapid accumulation of ubiquinated proteins in response to PR-957, a specific IP inhibitor.

Conclusion: Our results show that IP has a non-redondant role in the biology of cancer, more specifically in AML, and is associated to cancer survival. Moreover, an IP inhibitor specifically targets cells of M5 origin without affecting cells of M5 origin. Since specific IP inhibitors have now been synthesized by medicinal chemistry, tumors with high levels of IP could be targeted with minimal toxicity.

P022

Title: Role of nuclear exclusion of the deubiquitinase USP16 in coordinating DNA damage signaling

Authors: Nadine Sen Nkwe, Jessica Gagnon, Nicholas Iannantuono, Nazar Mashtalir, Natalia Zamorano, Erlinda Fernandez, Helen Yu and El Bachir Affar.

Affiliation: Dr. El Bachir Affar, Immunology-Oncology Department, Maisonneuve-Rosemont Hospital and Molecular Biology Programs, Université de Montréal

Keywords: Leukemia, Ubiquitination, Deubiquitinase, DNA repair, Histones

Background information : Histone H2A ubiquitination (H2Aub) is an epigenetic modification emerging as a central determinant in chromatin remodeling, which is prerequisite for various processes including cell proliferation and DNA repair. Deregulation of these pathways promotes leukemia development. The levels of H2Aub are regulated by enzymes called deubiquitinases (DUB) which remove ubiquitin from proteins. Indeed, recent studies identified USP16 enzyme as a regulator of Hox gene expression through H2A deubiquitination. Consistent with an important role in cell function, USP16 gene mutations encoding abnormal protein fusions has been identified in Chronic Myelomonocytic Leukemia (CML). Previous studies indicated that USP16 is predominantly cytoplasmic, but yet exerts nuclear functions. The mechanism that coordinates USP16 nucleo-cytoplasmic transport remains to be defined.

Purpose of the study: The aim of our research is to understand the mechanisms by which USP16 coordinate H2A ubiquitination and how defects in this pathway promote leukemia development

Methods: In these studies, we used molecular biology and cellular approaches applied to normal or tumoral cells. We employed RNAi to inhibit USP16 and analyze the molecular and cellular consequences. We also investigated by immunofluorescence the subcellular localization of USP16. In addition, we mutated various important domains of USP16 and determine the impact of these mutations on its localization, H2A deubiquitination and DNA damage response.

Results: Our studies showed that USP16 is mainly cytoplasmic suggesting that nucleo-cytoplasmic transport can play a crucial role in coordinating the function of this DUB in the nucleus. Indeed, we identified a nuclear localization signal (NLS) that when fused to GFP can promote its translocation into the nucleus. Following Leptomycin B treatment, an inhibitor of nuclear export, we observed a protracted accumulation of USP16 in the nucleus, indicating that this DUB is actively excluded from the nucleus by the CRM1/

exportin1 system. We note that when USP16 is translocated into the nucleus, H2Aub levels were drastically reduced. We also defined a mechanism whereby a strong nuclear export signal (NES) is responsible for the cytoplasmic retention of USP16. Our data also revealed that USP16 lacking the NES strongly abolishes the double strand DNA repair pathway through downregulation of the DNA damage signaling and ubiquitin ligase RNF168.

Conclusion: Our results provide insights into the complex nature of USP16 subcellular localization and its role in inhibiting H2A ubiquitination and DNA damage response. This study significantly contributes to the understanding of how the ubiquitin system coordinates DNA repair pathways. As the USP16 gene is translocated in leukemia, our study might help identifying novel inhibitors for the treatment of this disease.

P023

Title: *Ezh1* and *Ezh2* are essential regulators of normal and leukemic hematopoietic stem cells

Authors: Camille Simon

Affiliation: Dr. Guy Sauvageau, Molecular Genetics of Stem Cells laboratory, IRIC, UdeM

Keywords: EZH2, PRC2, Leukemia, Epigenetic, Hematopoiesis

Background: A growing number of evidence predicts similarities in the genes that regulate self-renewal of normal and cancer stem cells. Promising candidates include the Polycomb group (PcG) family of genes, which play a role in both stem cell self-renewal and in cancer. The canonical polycomb PRC2 complex, which displays methyltransferase activity on lysine 27 of histone H3, contains two alternative catalytic subunits: EZH1 or Ezh2. It has become evident that in various blood cancers, the PRC2 genes are frequently mutated, suggesting that defective PRC2 activity can be fundamental in the aetiology of haematological malignancies. Conversely, other blood cancers such as AML and B-ALL require an intact PRC2 complex to maintain their leukemogenecity.

Purpose of the study: The aim of this study is to understand the function of the catalytic subunits of the PRC2 complex, namely Ezh1 and Ezh2, in normal hemopoietic and leukemia stem cells. We put a focus on the identification of redundant functions between these two subunits.

Methods: We recently generated an *Ezh2/Ezh1* (Ezh1/2) conditional double KO (DKO) mouse model in order to completely abrogate the catalytic activity of the PRC2 complex in the hematopoietic cells. We used competitive transplantation assays to evaluate the self-renewal and the proliferation potential of mutant hematopoietic stem cells. We also performed rescue-type experiments with mutant *Ezh2* constructs in order to determine which domains are essential to its function. Finally, we retrovirally transduced hematopoietic cells from transgenic animals with *Hoxa9 and Meis1* oncogenes evaluated theirs ability to induce myeloid leukemia in recipient animals.

Results: While *Ezh1* or *Ezh2* inactivation alone does not induce a dramatic effect (*Ezh2* inactivation is mainly affecting B and T cell differentiation while *Ezh1* inactivation is slightly detrimental for hematopoietic stem cells self renewal), concomitant inactivation of *Ezh1/2* in the blood system leads to aplastic anemia and bonne marrow failure within two weeks. Competitive assays have shown that hematopoietic stem cell originating from double KO mice are not able to compete with their wild type counterparts and will rapidly disappeared from the recipient animal. Re-introduction of wild type Ezh2 but not a catalytic dead mutant is able to completely

restore cell survival. Finally, *Ezh1/2* co-inactivation is able to greatly delay the *HoxA9 Meis1* leukemia onset in comparison with *Ezh2* inactivation alone, while *Ezh1* inactivation do not give any advantages compared to WT *HoxA9 Meis1* leukemia.

Conclusion: Together, these studies show that Ezh1/2 are essential regulator of normal and leukemic hematopoietic stem cells, likely through their methyltransferase activity. We also showed that EZH1 and EZH2 share redundant functions essential for bone marrow homeostasis and cell survival.

P024

Title: A new marker for Acute Myeloid Leukemia with poor prognosis

Author: Clarisse Thiollier

Affiliation: Guy Sauvageau and Josée Hebert, IRIC, Université de Montréal

Keywords: Leukemia; complex karyotype; prognostic tool; marker of poor prognosis

Background information: Acute myeloid leukemia patients with complex karyotype (≥3 abnormalities) represent 20% of AML and are generally associated with poor outcome and reduced 3-year overall survival. It is a heterogeneous subgroup with patients carrying various mutations and chromosomal abnormalities. p53, considered as the "guardian of the genome", is found mutated in about 50% of these patients. Diagnostic methods need to be improved to predict how these patients should be treated according to their genetic characteristics.

Purpose of the study: As part of the LEUCEGENE project initiated at IRIC we analyzed 78 complex karyotype AML primary samples on different aspects (mutational status, cytogenetics, gene expression profiles) in order to refine the complex karyotype AML classification and improve the diagnostic tools and thus the treatment orientation of these patients.

Methods: Genetic abnormalities and transcriptome data were determined by next generation sequencing for 78 AML primary samples with complex karyotype (from BCLQ: Banque de Cellules Leucémiques du Québec) including 38 p53-mutated samples and correlated with clinical data. Mutations were validated by Sanger sequencing.Transcriptome data of these patients were compared to 337 non-complex AML from the BCLQ. Cells were subjected to several drug treatments and apoptosis induction was assessed by a luminescence assay (Caspase 3/7 Glo Assay, Promega). HCT116 cells were infected with a lentiviral vector for expression of GENE1 and subjected to the same tests.

Results: AML patients with complex karyotype form a heterogeneous subgroup with various genetic abnormalities. The most recurrent mutation is p53 found in about 50% of the patients, followed by RUNX1 mutation. No new recurrent mutation was identified. Looking at the transcriptional signature of these patients we identified GENE1 as amarker for complex karyotype AML and more generally as a marker of poor prognosis in AML. About 30% of the patients from our whole cohort (n=415) express a high level

of GENE1. This subgroup is highly enriched in complex karyotype and p53-mutated AML and shows a drastic reduction of overall survival rate. We observed that p53-mutated patients show a specific resistance to apoptosis induced by high doses of 5-fluorouracil that could not be associated to high expression level of GENE1.

Conclusion: We describe a large cohort of complex karyotype AML patients. We identified GENE1 as a marker of poor prognosis in a subgroup of patients highly enriched in complex karyotype. This population of AML shows a significantly reduced overall survival. The detection of this marker in AML would allow identifying 30% of patients that do not respond to standard treatments and who could benefit from investigational approaches.

P025

Title: Deciphering the Role of PP2A in Translation Control in Leukemia

Authors: Peng Wang, Paul Lasko, Nahum Sonenberg.

Affiliation: Laboratory of Dr Nahum Sonenberg, Department of Biochemistry, McGill University.

Keywords: Leukemia, Translation, S6K, 4E-BP, PP2A.

Background information: The protein phosphatase PP2A activity is commonly impaired in different types of leukemia. In animal models, restoration of PP2A activity selectively suppressed the survival and self – renewal leukemia cells but not quiescent hematopoietic stem cells. Remarkably, PP2A activating drugs have been demonstrated to antagonize leukemogenesis both *in vitro* and *in vivo*. On the other hand, mTOR pathway is a significant contributor to many human cancers, including hematologic malignancies. PP2A counteracts mTOR by dephosphorylating its two main substrates – S6K and 4EBP, which play crucial role in translational control. However, more than 70 PP2A holoenzymes can be assembled by combination of different subunit forms, which have the different substrate specificity. Therefore, identification of the specific forms of PP2A which counteract mTOR is required for better understanding of the translational control machinery in leukemia.

Purpose of the study: This work aims at identification of the specific forms of PP2A dephosphorylating S6K and 4EBP, and their interactions will subsequently be investigated in leukemia cells. I expect that my work could provide new and more specific leukemia therapeutic targets.

Methods: All PP2A regulatory subunits, which confer PP2A holoenzyme substrate specificity, will be cloned and expressed in cells. Then, physical association between S6K or 4EBP and specific regulatory subunit of PP2A will be examined by co-immunoprecipitation (co-IP) assay. Subsequently, phosphatase assay will be performed to further confirm the dephosphorylation of S6K or 4EBP by their associated specific form of PP2A. Then, the functional study of these interactions will be tested in leukemia cells by varying the expression of PP2A-S6K or 4EBP. Moreover, as dynamic localization of PP2A, S6K and 4EBP have been reported, their relative localization will also be investigated with high resolution microscopy.
If I can confirm that specific forms of PP2A regulate leukemia cells proliferation and differentiation through regulating S6K or 4E-BP, I will explore the possibility of pharmaceutical application of these specific forms of PP2A in leukemia.

Results: I found that PP2A-B55, but not PP2A-B56, can associate with 4EBP both in fly cells and mammal cells. Moreover, B55 putative substrate binding site mutation significantly decreases PP2A-B55 and 4EBP association. Furthermore, in fly ovaries where PP2A-B55 expression was decreased, phosphorylated 4EBP was dramatically increased. These results indicate that PP2A-B55 is the specific form of PP2A for 4EBP dephosphorylation. Further phosphatase assay of 4EBP dephosphorylation by PP2A-B55 is in preparation.

Conclusion: My results indicate that different specific form of PP2A may be required for dephosphorylating different translational factors such as S6K and 4EBP, which have been demonstrated to play crucial role in proliferation and differentiation of leukemia cells. Thus, further study of the precise molecular mechanism linking PP2A with S6K and 4E-BP can contribute to a better understanding of the translational control machinery in leukemia.

P026

Title: eIF4E coordinately upregulates Hyaluronic acid synthesis and production of its receptor CD44 to increase aggressiveness of AML cells

Authors: Hiba Zahreddine[1], Biljana Culjkovic-Kraljacic[1] and Katherine LB Borden[1*]

Affiliation: [1] Institute for Research in Immunology and Cancer & Department of Pathology and Cell Biology, University of Montreal, Qc, Canada

Keywords: eIF4E, CD44, HA synthesis, AML

Background: The eukaryotic translation initiation factor eIF4E is an oncogene that controls gene expression through its effects on mRNA export and translation. This requires that eIF4E binds the m7G cap on the 5' end of mRNAs which is key to its oncogenic potential. eIF4E is elevated in many cancers including M4 and M5 acute myeloid leukemia (AML) where its increased expression is associated with poor prognosis in patients.

Purpose of the study & Methods: To better understand the mechanisms underlying eIF4E's activities in proliferation, survival, invasion and metastases, we carried out two complimentary screens to identify candidate eIF4E export targets. The first screen was an RNA immunoprecipitation (RIP) from the nuclear fraction using an anti-eIF4E antibody and identifying associated RNAs using microarray (RIP-Chip). And the second screen determined which RNAs accumulated in the nucleus upon inhibition of eIF4E dependent mRNA export with an m7G cap competitor, ribavirin (fractionation-Chip). Analysis of our data strongly suggest that eIF4E coordinately modulates the expression of several key enzymes in the hyaluronic acid (HA) synthesis pathway as well as the major HA receptor, the integral membrane protein CD44. HA is a complex polysaccharide with differential functions based on chain length. Shorter forms of HA are primarily synthesized by hyaluronan synthase 3 (HAS3), which we show is an eIF4E target, and are associated with malignant phenotypes. HA plays critical roles as both a structural and regulatory component in the bone marrow extracellular matrix with its effects mainly mediated via CD44. Interactions of HA with its receptors can trigger intracellular signalling leading to inhibition of apoptosis in AML and T cell lymphoma, enhanced proliferation and migration. Additionally, CD44 agonists have been show to impair bone marrow homing and lodgment of AML and CML stem cells. Taken together, we *hypothesize that eIF4E overexpression confers a distinct proliferative advantage for AML cells by enhancing association with bone marrow through the effects of eIF4E on HA synthesis and CD44 expression*.

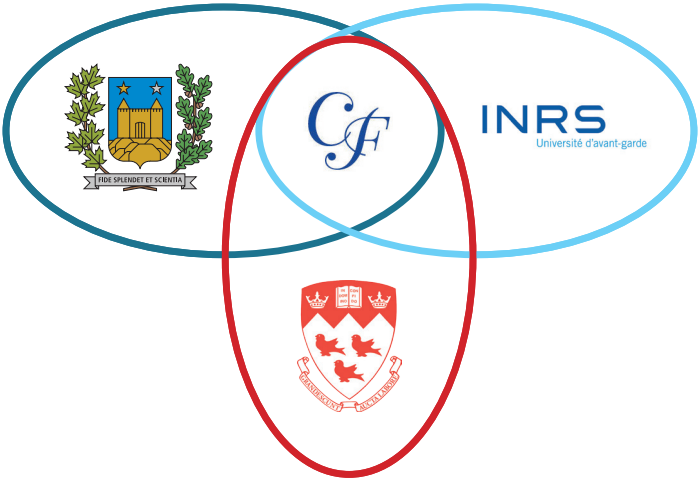
Results: To assess the effects of eIF4E on the synthesis of HA and the expression of CD44, we performed subcellular fractionation/qPCR on U2OS cells overexpressing eIF4E and on THP-1 ribavirin treated versus untreated cell lines to validate the RIP-Chip and fractionation-Chip results. RNAi knockdown of eIF4E in THP-1 cell line will be performed to confirm these results. Importantly, enzyme elevation does not prove that production of HA is also elevated. Accordingly, HA levels were measured in U2OS overexpressing eIF4E or vector control using fluorophore assisted carbohydrate electrophoresis. Our preliminary results revealed a two fold increase in HA levels in eIF4E overexpressing cells compared to control. This was confirmed by confocal microscopy using biotinlyated HA binding probes. As a functional output, we monitored transmigration activity as a function of eIF4E status to assess its effects on invasion. Using matrigel invasion chambers we show that eIF4E overexpression leads to increased cell migration and this was reversed by CD44 inhibitors and confirmed by RNAi-mediated knockdown of CD44.

Conclusion: Our findings will provide unique insights into the ability of eIF4E to increase invasion and metastases in solid tumours and likely underlies the aggressive nature of AML characterized by elevated eIF4E.

Notes

Notes

Notes


FIDE SPLENDET ET SCIENTIA
INRS
Université d'avant-garde