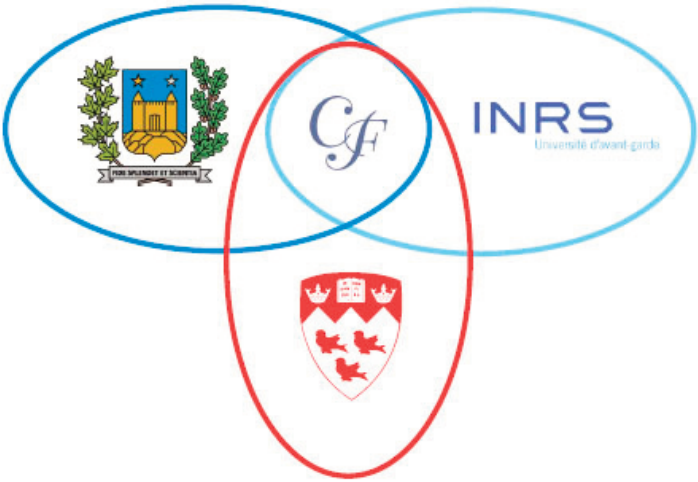
Cole Foundation
"Research Celebration Day"
May 9, 2014



Journée
« Célébrons la recherche »
de la Fondation Cole
9 mai 2014

The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD programmes to promote research in pre-leukemia, leukemia, and other leukemia-related diseases in children and young adults, as well as the development of clinical care for patients affected by these diseases. With the announcement of 2014 Fellows, the Fellowship programme has supported more than 115 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 $6 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.

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 postdoctoraux et des étudiants diplômés inscrits à des programmes de doctorat. Avec l'annonce des boursiers 2014, le programme a appuyé plus de 115 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 six 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'œuvre 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 - 10:30 AM	Round Table (new fellows) *Holmes Hall, 3605 de la Montagne Street*
10:00 AM - 11:00 PM	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 *Martin Amphitheater, McIntyre Building*
Welcome	*Dr. Elaine Davis* *Associate Dean, Biomedical BSc,* *Graduate and Postdoctoral Affairs, McGill*
New Research - New Realities Presentations	*Dr. Victor Ling* *Terry Fox Research Institute* *"The Future of Cancer Research: a Terry Fox Research Institute Perspective"*
	Dr. Hélène Decaluwe *Sainte-Justine Research Centre* *"Controlling T Cell Exhaustion to Prevent Leukemia Relapse"*
	Dr. Sonia Cellot *Sainte-Justine Research Centre* *"Histone Demethylases as Modulators of Hematopoietic Stem Cell Fate"*
	Dr. Kolja Eppert *Montreal Children's Hospital Research Institute* *"Regulators of Leukemic Stem Cells Linked to Patient Outcomes"*

2:00 - 3:15 PM

Introduction	*Dr. Nada Jabado* *Associate Professor, Department of Pediatrics* *McGill University*
Keynote Speaker	*Dr. Charles Mullighan* *Professor, Department of Pathology* *St. Jude Children's Research Hospital*
	"The Acute Lymphoblastic Leukemia Genome: Insights into Pathogenesis and Therapy"
3:15 - 3:30 PM	New fellows and announcement of prizes
3:30 PM	Reception

The 2014 - 2016 Cole Foundation Fellows
Les boursiers de recherche de la Fondation Cole 2014 - 2016

McGill University

Qianyu Guo, PhD program
Supervisor: Wilson Miller, Jr., Lady Davis Institute, Experimental Medicine
Project title: Elucidate the mechanisms of the increased anti-leukemic potency of darinaparsin compared to arsenic trioxide
Description: Current treatments for acute myeloid leukemia (AML) only cures ~50% patients. Despite its success in acute promyelotic leukemia (APL), arsenic trioxide (ATO) has limited effects in other types of AML. Darinaparsin (DAR) is a novel arsenical compound with increased potency against AML cells. However, it remains obscure why DAR is superior to ATO. I propose to compare and contrast DAR and ATO to provide information on future development of more powerful arsenical chemotherapeutic reagents.

Rayelle Itoua Maiga, PhD program
Supervisor: Jerry Pelletier, McGill University, Full Professor, Biochemistry
Project Title: Engineering Chromosome Translocations to Recapitulate Primary Events of CML
Description: Chromosomal rearrangements such as translocations play essential roles in tumor biology. The aim of this project is to use CRISPR/Cas9-based genome editing technology to induce double-strand breaks in chromosomes of hematopoietic stem cells to model specific known translocations. This will provide insight into the biology of these lesions and their role in cancer initiation.

Said Izreig, PhD program
Supervisor: Russell Jones, McGill University, Assistant Professor, Physiology
Project Title: Repression of LKB1 by miR-17/20 contributes to Myc-driven metabolism in lymphoma
Description: Cancer is a disease characterized by uncontrolled cell proliferation. At the cellular level, cancer cells adapt their metabolism to match the increased energetic demands of malignancy. My project explores the molecular basis for altered metabolic activity in aggressive lymphomas, with a focus on targeting cancer cell metabolism for therapeutic benefit.

Peng Wang, Post PhD program
Supervisor: Nahum Sonenberg, Rosalind & Morris Goodman Cancer Centre
Project Title: Deciphering the Role of PP2A-855 Localization in Translation Control in Leukemia
Description: The protein phosphatase PP2A activity is commonly impaired in different types of leukemia. Remarkably, PP2A activating drugs can efficiently antagonize the leukemogenesis both in vitro and in vivo. My project aims at deciphering the role of the spatiotemporal regulation of PP2A in leukemia cells.

Université de Montréal

Haithem Barbour, PhD program
Supervisor: El Bachir Affar, Centre de recherche de l'Hôpital Maisonneuve-Rosemont
Project Title: Deregulation of DNA methyoation in pediatric hematological malignacies
Description: TET2 is a major tumor suppressor gene mutated in pediatric leukemia. TET2 interacts with several proteins, including the transcription regulator OGT, ensuring proper hematopoiesis. Characterizing the defect of interaction between TET2 and OGT in childhood hematological malignancies will provide novel approaches for both diagnosis and treatment of the disease.

Nadine Sen Nkwe Dibondo, PhD program
Supervisor: El Bachir Affar, Centre de recherche de l'Hôpital Maisonneuve-Rosemont
Project Title: Dérégulation de l'ubiquitination de l'histone H2A dans les leucémies des enfants et des jeunes adultes.
Description: Ubiquitination is a biochemical process that plays a central role in blood cell formation and function. We are investigating how deregulation of histone H2A ubiquitination and deubiquitination promotes uncontrolled expression of Hox genes and juvenile leukemia. Our study will help designing improved diagnosis and therapeutic strategies for these childhood malignancies.

Nicolas Garnier, Post PhD program
Supervisor: Daniel Sinnett, CHU Centre de recherche Sainte-Justine
Project Title: Targeted therapy strategy for childhood ALL
Description: The establishment of the genomic landscape of pediatric pre-B ALL has revealed a complex architecture composed of a plethora of somatic mutations. We intend to pinpoint key driver mutations via a high throughput functional genomics approach. The identified actionable mutations might provide alternative therapy to affected children.

David Kachaner, Post PhD program
Supervisor: Vincent Archambault, IRIC, Biologie
Project Title: Inhibition of Polo-like kinase 1 interactions to disrupt mitosis in leukemia cells
Description: Several cancer cells types, including leukemia cells, have been shown to depend on elevated Polo-like kinase 1 (Plk1) activity for their proliferation and survival. My project consists in the characterization of novel Plk1 inhibitors that we have recently identified. These compounds will then be tested for their ability to selectively kill leukemia cells.

Vincent-Philippe Lavallée, Clinician program
Supervisor: Josée Hébert, Centre de recherche de l'Hôpital Maisonneuve-Rosemont
Project Title: Development of a novel molecular classification for improved risk stratification of AML patients based on next-generation sequencing.
Description: Based on next-generation RNA sequencing of a large cohort of AML samples from diverse cytogenetic groups, we will develop a statistical model combining selected mutations and gene expression that are associated to clinical outcome. This novel molecular classification aims to improve risk stratification and treatment of AML patients.

Valérie Lemay, PhD program
Supervisor: Daniel Curnier, Université de Montréal, Professeur agrégé, Kinésiologie
Project Title: Modifications à long terme des paramètres cardiorespiratoires chez des survivants de leucémie lymphoblastique aiguë: lien avec la pratique d'activité physique
Description: Les objectifs de mon projet sont: a) étudier les limitations cardiaques, pulmonaires et/ou musculaires à l'effort chez les survivants de leucémie lymphoblastique aiguë (LLA); b) étudier les liens entre la pratique d'activité physique des survivants LLA au moins 5 ans après leur rémission et l'état structurel et fonctionnel de leur coeur au repos et à l'effort. Ultimement, ces travaux mèneront à la mise en place d'interventions individualisées en activité physique visant à minimiser les effets secondaires à long terme qui sont observés chez les survivants LLA (cardiotoxicité, troubles métaboliques, obésité, ostéoporose, etc.).

Alena Motorina, PhD program
Supervisor: Frédérick Mallette, Centre de recherche de l'Hôpital Maisonneuve-Rosemont
Project Title: Understanding oncogene cooperation in pediatric acute megakaryoblastic leukemia.
Description: The cellular response to chromosomic fusions involved in pediatric acute megakaryoblastic leukemia is poorly understood, and the contribution of collaborating oncogenes activation and tumor suppressor inactivation required for tumorigenesis remains unknown. Our project aims at understanding the sequence of events bypassing the cell's anti-tumor defenses and causing acute megakaryoblastic leukemia.

Alexandre Rouette, PhD program
Supervisor: Claude Perreault, IRIC, Médecine
Project Title: Le rôle de l'immunoprotéasome dans l'oncogenèse
Description: Large-scale analysis of the involvement of proteasome's catalytic subunits in the genesis, progression and persistence of acute myeloid leukemia and other cancer types, using genomic and transcriptomic analyses of human clinical samples.

Jérôme Roger, PhD program
Supervisor: Philippe Roux, IRIC, Pathologie et biologie cellulaire
Project Title: Investigating the role of RSK in acute myeloid leukemia growth and chemoresistance.
Description: Flt3 is one of the most frequently mutated genes observed in acute myeloid leukemia (AML). These mutations cause constitutive activation of the protein kinase RSK, which we found inhibits the tumour suppressor p53. In this project, we will determine if the regulation of p53 by RSK promotes AML chemoresistance.

Clarisse Thiollier, Post PhD program
Supervisor: Guy Sauvageau, IRIC, Médecine
Project Title: Inactivation de p53 dans les leucémies aigües myéloïdes
Description: Je m'intéresse à l'identification et à la caractérisation des inactivations du facteur p53 chez les patients atteints de LAM, par une approche de chemogénomique. Ce travail permettra d'améliorer la classification des LAM et la prise en charge des patients présentant un facteur p53 inactif.

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

Presenters / Liste des exposants

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Assaker, Gloria
Bolt, Alicia
Celton, Magalie
Dupéré-Richer, Daphne
Fang, Yi
Gerby, Bastien
Grapton, Damien
Guégan, Jean-Philippe
Maiga, Arhama
Mancini, Johanna
Montpas, Nicolas
Oussa, Eustache
Pabst, Caroline
Robellet, Xavier
Simon, Camille
Tsao, Sarah
Veiga, Diogo
Zahreddine, Hiba

Poster List / Liste des projets exposés

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PO3	Bolt, Alicia	Investigating the Effects of Tungsten on Bone Marrow and the Bone Marrow Microenvironment
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PO9	Guégan, Jean-Philippe	TC21 (R-ras2), a new MAP kinase ERK1/2 substrate
PO10	Maiga, Arhama	Expression analysis of GPCR in acute myeloid leukemia
PO11	Mancini, Johanna	Selective Telomere Shortening in Cancer Cells by a G-quadruplex Ligand
PO12	Montpas, Nicolas	Role of CXCR7 as a negative modulator of leukemia cells in bone marrow niche homing
PO13	Oussa, Eustache	Importance of TRAF1 phosphorylation on the recruitment of TBK1 in CD8 T cells
PO14	Pabst, Caroline *(absent)*	Identification of Small Molecules That Support Human Leukemia Stem Cell Activity *Ex Vivo*
PO15	Robellet, Xavier	A Cdk1-Dependent Phospho-Switch to Initiate Mitotic Chromosome Condensation
PO16	Simon, Camille	*Ezh2* is an essential regulator of T-cell development and oncogenic transformation in mouse and human adult T cell acute leukemia
PO17	Tsao, Sarah	Inhibition of histone deacetylation as a novel antifungal therapy in leukemia patients

PO18	Veiga, Diogo	Self-renewal network activated by SCL-LMO1 confer aberrant stem cell properties to immature thymocytes
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PO1

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

Authors: Mohamed Aissiou and Clémence Balosetti

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

Keywords: doxorubicin cardiotoxicity, cine MRI, myocardium biomechanics, acute lymphoblastic 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 tracking myocardial 3D deformation and mechanics. 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 study aim was to propose an imaging-based method to quantify myocardial deformation and mechanics using clinical Cine MRI. The hypothesis is that doxorubicin chemotherapy induces direct damage to myocardial fibers architecture which can be detected using finite-element-based biomechanical modeling.

Methods: We used an ECG-gated Cine MR sequence to image the whole heart in a complete cardiac cycle in 5 adult volunteers. Acquisition parameters were slice thickness 5 mm, matrix 256 × 256, pixel spacing 1.25 × 1.25 mm and cardiac delay time 40 ms. A total of 10 slices per plane (including short-axis, 4-chamber and 2-chamber views) and 30 phases of the cardiac cycle per slice were obtained during multiple breath-holds of 10-15 s. The overall acquisition time including the 3-plan localizer scan was approximately 25 minutes. Myocardial wall contours were extracted and tracked at each phase and parameterized using per-length normalization using a custom-written Matlab (The MathWorks, Inc.) program. The 3D displacement at each point was assembled using component-by-component assembly method and injected to a reconstructed model of the ventricles.
Displacement errors were estimated between the reconstructed model (reference) at mid-systole (MS=200ms) and the simulated model, which was obtained by adding generated 3D displacements to ED (end-diastole) model (ED=0ms).

Results: Estimated RMS errors between MS model and simulated MS were 3.2 ± 2.97 mm. However, local errors of up to ~7 mm were detected. The qualitative distribution of deformation and stresses within the myocardium seems in agreement with Tagging MRI reported data. However, a small sliding phenomenon was noted, which we believe is due to 2D curve parameterization.

Conclusion: Deformation patterns analysis successfully allowed a personalized reconstruction and tracking of the 3D myocardial deformation. We believe that the use of future refined mesh would significantly improve displacements estimation. However, one limitation to this technique remains related to intramyocardial deformation which is not taken into account.

PO2

Title: Identification of novel regulators of the Notch ligands Delta-Like 1 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. Hence, understanding how Notch signaling is regulated is central to understand cancer development and to identify new potential therapeutic targets. 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 ligand Delta activity that subsequently modulate Notch signaling and its oncogenic activity in T-ALL.

Methods: To unravel new Delta regulators, we performed a genome-wide shRNA screen using an in vitro co-culture assay, and targeting specifically the signal-sending cells. We used the OP9 mouse stromal cells stably expressing the ligand Delta-like 1 (DL1) as signal-sending cells, and HeLa cells stably transfected with a luciferase reporter of Notch as signal-receiving cells. This screen allowed us to identify 166 primary hits, which were further confirmed in OP9 cells expressing another DSL ligand: DL4. Using functional secondary assays, we found that those hits are required for Notch-mediated events such as differentiation of hematopoietic progenitors into T-cells, and maintenance of pre-leukemic stem cells (pre-LSCs) isolated from a T-ALL mouse model. Moreover, we showed that some newly identified regulators are necessary for the progression of pre-leukemic T-cells into leukemic T-cells in vivo, following engraftment.

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. Interestingly, among the validated hits, we found genes that are known to be 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: Overall, this study led to the identification of novel DL1/4 regulators that could serve as potential therapeutic targets in Notch cancers, as exemplified by the T-ALL model. While some Notch inhibitors are currently in clinical trials, they present very limited options due to their gastrointestinal toxicity and their weak anti-leukemic effects against human T-ALL, highlighting the need for alternative strategies.

PO3

Title: Investigating the Effects of Tungsten on Bone Marrow and the Bone Marrow Microenvironment

Author: Alicia Bolt

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

Keywords: Tungsten, Bone Marrow, Hematopoiesis, Mesenchymal stromal cells, 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 have been 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 wild-type mice in a concentration-dependent manner, making the bone and bone marrow microenvironments potential sites of tungsten toxicity. The purpose of this study was to define what effects tungsten has on bone marrow and the bone marrow microenvironment.

Results and Conclusions: Mice exposed to tungsten in drinking water over a 16-week exposure have altered B-cell development and increased DNA damage within the bone marrow. Tungsten-exposed mice have an accumulation of pre-B cells at 16 weeks, the same B-cell stage commonly found in pediatric leukemia. We have developed a 4-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 will be determined by RNA-Seq. In addition, we are using an inducible TEL-AML1 mouse model of leukemia to evaluate if tungsten can provide one of the 2-hits required for leukemogenesis. Based on the initial set of mice analyzed, 6 of 9 tungsten-exposed, TEL-AML1 mice developed some type of disease pathology with 44.4% developing splenomegaly. While splenomegaly is not a definitive marker of leukemia, these are promising results that suggest that tungsten can induce immune pathologies in this model that we are currently characterizing. Tungsten accumulation in the bone is associated with direct effects on hematopoietic cells but may also indirectly affect hematopoiesis by targeting resident stromal cells in the bone marrow. Mesenchymal stromal cells (MSC) support hematopoiesis, but also differentiate into osteoblasts, the cells responsible for bone formation, or adipocytes.

This dynamic balance between osteoblast and adipocyte formation has also been shown to influence hematopoiesis. We tested whether tungsten changed primary murine MSC differentiation in vitro in the presence or absence of tungsten. Tungsten skewed MSC differentiation by decreasing osteoblast markers and increasing adipocyte markers. In vivo, tungsten increased expression of adipocyte markers in murine bone marrow by 4 weeks, but did not change expression of osteoblast markers. These data indicate tungsten alters MSC, which may result in changes in hematopoiesis and immune pathologies we observed.

PO4

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 identify 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 the understanding of the evolution of gene expression patterns during stages of leukemogenesis by highlighting the involvement of DNA methylation in gene expression changes.

Methods: We have sequenced DNA and RNA from 3 pediatric MA9 AML patients but could not identify novel common mutations. This first analysis showed that patient genetic heterogeneity is a considerable challenge. We therefore developed, in collaboration with Dr. Fred Barabé, a novel model system using primitive human hematopoietic cells expressing a human MLL fusion gene transplanted into immunodeficient mice, generating AML, with features that recapitulate human leukemia. To study the DNA methylation patterns, we use a recent protocol combining DNA bisulfite treatment with an in-solution hybrid capture procedure provided by the Agilent SureSelect target enrichment system to survey DNA methylation in CpG islands genome-wide.

Results: Using our experimental system, we performed RNA sequencing at each step during the evolution of the leukemias. The correlation between the data from two different donors and two pediatric MA9 AML patients showed that the use of the AML model provides reduces the problem of variable genetic backgrounds. We identified candidate genes specific to MA9 AML by the selection of genes expressed in MA9 AML samples but silenced in other subtypes of AML and in non-leukemic cells. We next generated bisulfite-converted NGS libraries from the DNA of CD34+ enriched cord blood cells and CD34+ cells transduced with human MA9 fusion gene (CD34+-MA9 AML). We have now started to analyze over 200 million individual methylated cytosines and compared the relevant regions differentially methylated between the normal cord blood and the CD34+-MA9 AML. We will specifically look at genes whose expression changes are correlated to DNA methylation patterns in MA9 AML.

Conclusion: This new mouse model using single donor human cord blood cells provides an experimental system to remove the confounding effects arising from a mixed genetic background. The presence of abnormal DNA methylation levels plays a role in AML pathophysiology and by our approach we will define the potential mechanisms behind the DNA methylation patterns specific to MA9 AML.

PO5

Title: HDACi induces differentiation of DLBCL cells associated with sensitization to proteasome inhibition

Author: Daphné Dupéré-Richer

Affiliation: Dr. Wilson H. Miller Jr. lab, Lady Davis Institute

Keywords: HDAC inhibitors, lymphoma, resistance, autophagy

Background information: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoid malignancy in the western world (1), representing 30% of non-Hodgkin lymphoma. A large number of genes encoding epigenetic modifying enzymes are mutated in DLBCL, which implicates epigenetic regulation as an important factor in DLBCL pathogenesis, and a potential target for therapy. Among epigenetic therapies, histone deacetylase inhibitors (HDACi) have clinical activity in a subset of DLBCL patients, thus defining predictive biomarkers, and developing combination therapies is critical.

Purpose of the study: Aiming to understand the molecular effects of HDACi in DLBCL.

Methods: We developed HDACi-resistant cell lines from the SUDHL6 and SUDHL4 human cells by a dose escalation protocol. A Gene expression array analysis was performed in parental SUDHL6 and the resistant clone SUDHL6-X.

Results: Strikingly, we observed features of more differentiated, plasmablast-like cells in SUDHL6-X cell. These changes included inactivated B cell receptor signaling, increased activation of the unfolded protein response, metabolic reprogramming and a switch in gene expression profiles from GCB to ABC subtype and from BCR-dependent to independent. These characteristics were reflected by dramatically increased susceptibility to inhibitors of the proteasome bortezomib and MLN2238. Importantly, analysis of lymphoma cells isolated from de novo resistant DLBCL patients treated with the HDACi panobinostat for 15 days showed a profile of changes similar to our in vitro model, indicating that our observations are not exclusive to our in vitro models.

Conclusion: Our results highlights sensitization with HDACi as a strategy to treat DLBCL in the clinic, potentially in combination with proteasome inhibitors.

PO6

Title: Chimeric oncogenes as regulators of the vascular activity expressed by extracellular vesicles derived from leukemic cells

Author: Yi Fang

Affiliation: Dr Janusz Rak, Department of Pediatrics, Research Institute of Montreal Children's Hospital, McGill University Health Center

Keywords: extracellular vesicles, acute promyelocytic leukemia, PML-RARa

Background information: In pediatric leukemia oncogenic alterations affect not only intrinsic properties of cancer cells but also interactions between these cells and stroma. To study these processes we employed a paradigm of acute promyelocytic leukemia (APL) which is driven by a transforming fusion between promelocytic leukemia gene (PML) and retinoic acid receptor alpha(RARa). The oncogenic effect of PML-RARa relies upon RARa-dependent inhibition of cellular differentiation and can be selectively obliterated by all-trans retinoic acid (ATRA) therapy. This is paralleled by down regulation of the procoagulant receptor tissue factor (TF) and a number of vascular effectors, emitted from leukemic cells as cargo of extracellular vesicles (EVs), mediators of intercellular communication.

Purpose of the study: In this study, we explored the impact of ATRA and its target (PML-RARa) on EV emission and interaction of APL cells with vascular endothelium. We reasoned that ATRA-modified EVs may play a role in bone marrow angiogenesis and leukemic niche.

Methods: We employed human NB4 cells responsive to ATRA as a model of APL. Vesiculation of NB4 cells was profiled by Nanoparticle Tracking Analysis (NTA) and the vesicle cargo analyzed using mRNA profiling, RT-PCR, Western blotting and ELISA assays. Uptake of EV-related material by endothelial cells (HUVEC) was measured using PHK-26 staining and flow cytometry, while the resulting changes in cellular phenotype were monitored by TF procoagulant assays, growth bioassays and molecular profiling.

Results: Exposure of NB4 cells to ATRA causes cellular differentiation, alteration in the angiogenic profile and changes in cellular vesiculation. Leukemic EVs contain transcripts for both, PML-RARa oncogene, and several of its targets, such as TF and a series of key regulators of angiogenesis (VEGFR), as well as the related proteins. In line with these findings, the uptake of NB4-derived EVs confers functional changes on endothelial cells, including procoagulant phenotype and modulation of growth, survival and migration.

Conclusion: Oncogenic fusion genes, such as PML-RARa regulate the web of interactions between leukemic (APL) cells and their vascular niches. This process involves oncogene-driven emission of EVs and transfer of their cargo between leukemic and endothelial cells. PML-RARa protein is not detected in leukemic EVs, but exerts an indirect effect on their production and cargo. APL-derived EVs may render endothelial cells procoagulant and stimulate their proliferation. The significance of EV-mediated interactions between leukemic and non-leukemic cells is being investigated.

PO7

Title: SCL, LMO1 and Notch1 reprogram thymocytes into self-renewing cells

Author: Bastien Gerby

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

Keywords: SCL, LMO, Notch1, self-renewal, pre-leukemic stem cell.

Background information: Normal hematopoietic stem cells (HSCs), located in the bone marrow, are uniquely endowed with self-renewal activity. They are able to generate thymic progenitors devoid of this stem-cell property. These cells progress into the thymus through several stages of differentiation (DN1-4, ISP8, DP) before giving rise to CD4+ or CD8+ immunocompetent cells. SCL is a basic helix-loop-helix (bHLH) transcription factor that is crucial for the maintenance of HSC function. LMO1/2 belong to LIM domain proteins that associate with SCL in hematopoietic development and NOTCH1 pathway is indispensable for T-cell development in the thymus. Ectopic expression of SCL and LMO1/2 is found in almost 25% of childhood T cell acute lymphoblastic leukemia (T-ALL) and more than 50% harbour NOTCH1 mutations leading to the constitutive activation of the pathway.

Purpose of the study: Here, our goal was to explore the cellular and the molecular mechanisms by which the SCL, LMO1 and Notch1 oncogenes reprogram normal thymocytes. Precisely, we wanted to determine whether these three oncogenes induce stem cell-like properties to pre-leukemic thymocytes before T-leukemia development.

Methods: We took advantage of a transgenic mouse models that closely reproduce paediatric human T-ALL to define oncogenic events during the pre-leukemic phase. The aberrant stem cell-like properties of pre-leukemic thymocytes were assessed in vivo by transplantation assay and in vitro by co-culture.

Results: We show that the three oncogenes, SCL, LMO1 and Notch1 perturb the molecular network of normal thymocyte progenitors to convert them into pre-leukemic stem cells (pre-LSCs). Precisely, we provide evidence that SCL and LMO1 induce an aberrant self-renewal potential in DN3 thymocytes. At the molecular level, SCL directly interacts with LMO1 to activate the transcription of a self-renewal gene network in DN3 thymocytes that includes Lyl1, which can substitute for SCL. We next showed that DN3 are the cellular targets of reprogramming by SCL-LMO1 due to higher endogenous NOTCH1 activity, compared to other thymocyte subpopulations. Indeed, elevating NOTCH1 activity with the Notch1 oncogene results in a dramatic expansion of the pool of SCL-LMO1-induced self-renewing pre-LSCs. These results indicate that SCL-LMO1 reprogramming activity depends on NOTCH1 levels. Unexpectedly, Notch1 does not reprogram thymocytes by itself. Rather, Notch1 confers a proliferative advantage to SCL-LMO1-induced pre-LSCs. Our observations indicate that Notch1 levels determine SCL-LMO1 reprogramming activity in thymocytes which requires transcription activation by the SCL complex. Finally, our results indicate that thymocyte reprogramming is a major determinant of T-ALL.

Conclusion: The SCL-LMO1 oncogenes reprogram DN3 thymocytes to acquire self-renewal potential, thereby establishing a pre-leukemic state. NOTCH1 activation provides a strong signal that collaborates with the SCL-LMO1 oncogenes to induce T-ALL by favoring self-renewal divisions in pre-LSCs. Together, this work provides a rationale not only for future molecular cell biological studies of T-ALL stem cells but also for the development of therapeutic strategies that target the aberrant self-renewal function induced by oncogenes in thymocytes progenitors.

PO8

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

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

Background information: Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are haematological malignancies. AML is characterized by increased proliferation and impaired hematopoietic differentiation of myeloid progenitor cells in the bone marrow. MDS are often considered as pre-AML stage in which hematopoietic progenitors exhibit impaired differentiation and will eventually progress into AML. Our laboratory reported the existence of a single nucleotide polymorphism (SNP) in the gene GFI1, which leads to substitution of a serine by an asparagine at amino-acid position 36 (GFI136N). People with this variant GFI1 allele have a 1.6 times higher risk of developing AML. More recent data from our team suggest that GFI136N predisposes to myelodysplastic syndromes (MDS) and increases by 3 fold the risk of developing an AML from a pre-existing MDS.

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 GFI136S (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 will use a retroviral model where oncofusion-proteins such as AML1-Eto9a or MLL-AF9 are overexpressed in a progenitor-enriched population of mouse bone marrow that can mimic the AML development in transplanted recipient mice. 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.

Results: After retroviral transduction with AML1-Eto9a or MLL-AF9, transduced Gfi136N/36N cells generated more colonies with a 2-4 fold more cells/colonies in methylcellulose assays (p=0.05). We are now monitoring mice transplanted with cells from either Gfi136S/36S or Gfi136N/36N to see if the variant form accelerates the onset of the disease. NHD13 transgenic animals show the first signs of myelodysplastic syndromes by the age of 4-7 months and will eventually die of leukemia by the age 14 months. 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.

Conclusion: Our data suggest that Gfi136N is a novel predictive marker for AML development among MDS and AML patients. We are examining drugs targeting these epigenetic changes to treat MDS and AML patients.

PO9

Title: TC21 (R-ras2), a new MAP kinase ERK1/2 substrate

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Affiliation : Dr Meloche Sylvain, IRIC, Université de Montréal

Keywords: TC21, MAPK, signaling pathway, leukemia

Background information: The small GTPase TC21, also known as R-ras2, belongs to the Ras superfamily 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. The aim of these regulations are still under investigation but our preliminary results showed that this phosphorylation did not affect the basal localization of TC21, the stability of the protein or its activation by its main GEF. However, the phospho-mimetic mutant of TC21 seems to confer a mitogenic and/or pro-survival advantage. Overexpression of TC21 is sufficient to transform normal fibroblasts and we showed that the expression of the phospho-mimetic mutant of TC21 accelerate the growth of xenografts in nude mice.

Conclusion: The phosphorylation of TC21 by ERK1/2 could therefore represent a major mechanism of regulation of TC21 and its 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.

PO10

Title: Expression analysis of GPCR in acute myeloid leukemia.

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Keywords: GPCR, RNAseq, AML, transcriptome

Background information: Acute Myeloid Leukemia (AML) is the most deadly form of leukemia, with only 22.6 % of 5-years relative survival rate in the U.S. The current treatment of AML is based on chemotherapy (anthracycline and cytarabine) with or without transplantation of hematopoietic stem cells. However, this treatment is inefficient and the majority of patients will have significant side effects due to chemotherapy and / or will eventually relapse and die from this disease. It is therefore urgent to identify new potential therapeutic targets for the development of more effective and less toxic treatments of AML.
In search for such potential treatments, we focused on G protein coupled receptors (GPCR), which have an important therapeutic potential, accounting for 30% of all marketed drugs target. They represent the largest family of membrane receptors (~800 in human) and constitute key transducers for cells to adapt to their surrounding environment.

Purpose of the study: This project aims at identifying GPCR which expression is deregulated in AML when compared to normal hematopoietic progenitor cells as po
tential therapeutic targets.

Methods: We used RNAseq to evaluate the expression of 772 GPCR in 152 AML samples of the Québec Leukemia Cell Bank (BCLQ) and 12 normal hematopoietic progenitor cells sorted from cord blood provided by Hema Québec. The cohort of AML includes the following karyotype abnormalities: 19 t(8;21), 26 inv (16), 50 normal Karyotype (NK), 32 MLL translocation, 9 EVI translocation, 8 complex karyotype. The 772 GPCR analyzed here include GPCRs of the IUPHAR database as well as 370 olfactory, 24 taste and 4 vomeronasal receptors.

Results: Our analysis highlight 15 GPCR that are highly expressed in AML, with RNA level close to endogenous control genes such as GAPDH or ACTB. A comparison of GPCR expression between AML and normal cells showed that 30 and 19 GPCR are up- and down-regulated, respectively, the other GPCR being equally expressed between the two groups. Up-regulated GPCR are enriched in chemokine, Eicosanoid lipid, and Purine receptor family members whereas the down-regulated GPCR are enriched in adhesion, protease-activated and Frizzled. GPCR expression level analysis, with regard to the karyotype abnormalities and the gene mutations found in the cells, showed a significant difference of expression for some GPCR between t(8;21), inv (16) and MLL translocated, FLT3ITD, NPM1 and DNMT3 mutated samples as compared with the samples without these abnormalities.

Conclusion: Our results revealed a strong expression of some GPCR like CXCR4, CD97 and PTGER4 in all AML sequenced, highlighting these genes as potential targets for the development of AML therapeutics. Down-regulated and up-regulated GPCR in AML belong to specific receptor subfamilies thus pointing to potential roles of these receptors in disease ontogeny or progression. When considering karyotype abnormalities or mutations found in AML, a clear difference in some GPCR expression can be appreciated illustrating the heterogeneity of these different AML types and offering opportunities for the development of disease specific markers and/or therapeutic approaches.

PO11

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

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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 r ecombination-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, in telomerase-negative ALT, and in 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.

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.

Conclusion: Our results demonstrate that G-quadruplex stabilizing ligand PIP can significantly affect telomerase-positive cell proliferation and cause increased telomere attrition at a dilution of 0.5X IC50. Furthermore, our results suggest that the observed anti-proliferative effects are unlikely due to apoptosis, and we are currently assessing if these effects are due to senescence. Our studies will 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.lation. We also determined that CR is a conserved feature of AID, further promoting its importance in regulating AID localization. Interestingly, we observed that mouse AID did not respond strongly to leptomycin B. This was confirmed in mouse B cells, and is due to two residues in the C-terminus of mouse AID that increase hydrophobicity. Finally, we determined that though mutations affecting AID CR tend to cause a modest reduction in enzymatic and class switching activity, they cause up to 3-fold higher somatic hypermutation activity, confirming the importance of CR in regulating AID biological activity.

PO12

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

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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 minimal residual disease (MRD) that limited therapy success after chemotherapy and eventually lead to relapse. Indeed, the bone marrow microenvironment provides 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 the impact of CXCR7 expression on: 1- CXCR4-mediated migration, 2- CXCR4 re-sensitization capacity and 3- the aggressivity of a specific leukemia in a mouse model.

Methods: Our experiments have been performed on REH cell line (pre-B acute lymphoblastic leukemia (ALL) cell line; aims 1/2) or on bone marrow biopsies from patient diagnosed with ALL (aim 3). Aim 1/2: The overexpression of CXCR7 has been achived by the NucleofectorTM technology or by lenti-viral infection. Dose-response migration experiments have been accomplished on ChemotoX® Disposable Chemotaxis System and the migration potency determined by cell count. The expression of CXCR4 and CXCR7 has been assessed by flow cytometry using fluorochrome-coupled monoclonal antibody. Aim 3: We evaluated the time-to-leukemia parameter following intravenous injection of bone marrow biopsy from patient diagnosed with ALL in humanized NOD/SCID mice model. The expression if CXCR4/7 was previously characterised by flow cytometry in each sample graft in mices.

Results: For the first time, we 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. We also demonstrate that the presence of CXCR7 impinges the recycling capability/re-sensitization of CXCR4. This last results suggest that the fate of CXCR4 following CXCL12 stimulation would be in part controlled by the expression of CXCR7. Finally, preliminary results from the correlation between the expression of CXCR4/7 and the time-to-leukemia will be presented.

Conclusions: CXCR4-mediated chemotaxis/migration response results from a persistent receptor activation/intracellular signaling. Taken together, our results suggest that CXCR7 act as a negative migration modulator of CXCR4 - and thus bone marrow niches homing – by affecting its recycling/re-sensitization capability. The ratio of CXCR4 and CXCR7 expression in leukemia patients may reveal predictive for bone marrow homing, and thus for the risk of MRD and relapse.

PO13

Title: Importance of TRAF1 phosphorylation on the recruitment of TBK1 in CD8 T cells

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Keywords: Leukemia, Non-Hodgkin lymphoma, TRAF1, survival, proliferation

Background information: TNFR-associated factors (TRAFs) are adapter proteins involved in signaling downstream of members of the TNFRs family. Our laboratory has previously shown an essential role of TRAF1 in survival of memory T cells and demonstrated that TRAF1 was required downstream the 4-1BB receptor, a member of the TNFR family. Overexpression of TRAF1 has been observed in 48% of leukemia and lymphomas and its role is correlated with survival. A recent study by Kato Jr et al showed that PKN1 is able to phosphorylate TRAF1 (mouse on serine 139, serine 146 in human). Accordingly IKK and JNK activities are inhibited downstream of TNFR2. Indeed, we have recently demonstrated TRAF1 to be associated with TBK1. The majority of current knowledge on TBK1 is in the innate immune responses to viruses because TBK1 regulates the activity of interferon regulatory factors (IRFs) and the production of type I interferon. However, TBK1 has recently been associated with oncogenesis and proven to be essential to the survival of KRAS mutant tumours, demonstrating the role of TBK1 in cell survival. KRAS mutations in human hematological malignancies are often associated with myeloid disease and T cell proliferation. Additionally, the KRAS gene is mutated in 10% of patients with lymphoblastic leukemia and lymphoma. However, the functional importance of TBK1 and its association with TRAF1 in the survival of healthy and malignant cells remains to be determined.

Purpose of the study: Our main objective was to characterize the cooperation between TRAF1 and its binding partners in the survival of haematological malignancies.

Methods: Murine TRAF1 WT and S139A mutant were introduced into the human leukemia Jurkat E6.1 cell line, the murine TRAF1 protein was immunoprecipitated and liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed. TANK and TANK binding kinase 1 (TBK1), two molecules involved in the activation of NF-κB downstream TNFRs were identified to be preferentially associated with TRAF1 S139A. TRAF1 has been shown to associate with TANK and TBK1 activity depends on TANK.

Results: Using a proteomics approach we demonstrate that TBK1 preferentially associates with the TRAF1 Serine 139 to Alanine (S139A) mutant. TBK1 is a kinase that functions upstream of NIK and IKK in the activation of the NF-κB pathway. When T RAF1-deficient CD8 T cells were reconstituted with the TRAF1 S139A mutant, we observed more sustained levels of IκBα degradation in response to 4-1BB stimulation in contrast to cells expressing either TRAF1 wild-type or TRAF1 S139D phospho-mimetic mutant. Together, these findings define the importance of the basal phosphorylation state of the TRAF1 Serine 139 residue in coordinating signalling events downstream of 4-1BB in primary T cells.

Conclusion: Our experiments reveal a novel insight into the impact of TRAF1 phosphorylation in fine tuning intracellular events by coordinating the recruitment of signalling mediators involved in NF-κB activation following 4-1BB co-stimulation in T cells. To confirm our results, paediatric samples from Quebec Leukemia Cell Bank will be used to assess the cooperation between TRAF1 and TBK1 and survival.

PO14 *(absent)*

Title: Identification of Small Molecules That Support Human Leukemia Stem Cell Activity *Ex Vivo*

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Keywords: AML, Leukemic Stem Cells, Self-renewal, Aryl-hydrocarbon Receptor

Background information: Therapy failure and relapse following treatment of acute myeloid leukemia (AML) are thought to be the consequence of self-renewing, chemotherapy resistant disease propagating Leukemic Stem Cells (LSCs) within the bulk of leukemic blasts. These cells are experimentally defined by their ability to engraft immunocompromised mice, and their frequency within leukemic clones has been associated with clinical outcome in adult and pediatric leukemia patients. Therefore, defining the pathways that control LSC self-renewal is a crucial prerequisite for the development of targeted therapies. Whereas normal human cord blood derived hematopoietic stem and progenitor cells can be successfully expanded in cytokine supplemented serum-free media in optimized systems, currently available culture conditions fail to promote self-renewal of their leukemic counterparts in the great majority of primary AML specimens.

Purpose of the study: The aim of this study was to identify small molecules and molecular pathways that promote the ex vivo expansion of undifferentiated primary human AML cells.

Methods: We tested ~6,000 chemicals for their ability to expand a primary human AML sample with normal karyotype in vitro while maintaining phenotypic, morphologic, and functional characteristics. Loss of CD34 expression and acquisition of the lineage marker CD15 were monitored by flow cytometry as an indication of ongoing differentiation. 17 genetically and morphologically diverse AML samples were subsequently exposed to the two AhR suppressors, SR1 and N-methyl-beta-carboline-3-carboxamide, as AhR antagonists were highly enriched among hit compounds. RNA-Seq and q-RT-PCR were used to analyze AHR and AhR target gene expression in fresh and cultured AML cells. Furthermore, all samples were exposed to the Pyrimido indole UM729 alone and in combination with AhR antagonists. To determine whether functionally engrafting LSCs were supported under in vitro conditions, we transplanted fresh and cultured AML cells into immunocompromised NSG mice.

Results: The AhR pathway is rapidly and robustly activated in primary human AML specimens following in vitro culture. Suppression of the pathway by small molecules allows for the expansion of primary human CD34+ AML cells and partially rescues their capacity to engraft immunocompromised mice. Furthermore, we identified a novel Pyrimido indole, UM729, which has an additive effect with AhR antagonists in preventing differentiation in most AML specimens. UM729 lacks AhR suppressor activity thus indicating that at least two different pathways contribute to the maintenance of LSC activity *ex vivo*.

Conclusion: Together, our results are an important step towards the establishment of defined culture conditions to overcome spontaneous differentiation and cell death observed in ex vivo cultures of primary human AML specimens. Our findings suggest that at least three molecular targets might be at play in this process, two of which are targeted by the compounds SR1 and UM729. These two compounds can be implemented as a standardized media supplement and will pave the way for studies of self-renewal mechanisms and for the identification of new anti-leukemic drugs through cell-based assays for primary human AML cells.

PO15

Title: A Cdk1-Dependent Phospho-Switch to Initiate Mitotic Chromosome Condensation

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Keywords: Condensin, Cell-cycle, Burkitt's lymphoma, Epstein Barr Virus

Background information: During cell division, chromatin undergoes drastic structural changes to ensure efficient partition of genomic DNA to daughter cells. The condensin complex orchestrates this reorganization leading to the formation of mitotic chromosomes. Despite its importance, little is known about the mechanism used by condensin to initiate DNA condensation and how this process might be regulated. Recently, it has been shown that a virus associated with the formation of Burkitt's lymphoma –the Epstein Barr virus (EBV)– encodes a kinase that phosphorylates condensin when infecting cells. Remarkably, this phosphorylation is associated with premature chromosome condensation and leads to genome instability, thereby providing a potential mechanism for the development of Burkitt's lymphoma in children and young adults. The EBV kinase seems to act in this process by phosphorylating condensin on residues that are normally phosphorylated by the cyclin-dependant kinase (CDK) during mitosis. The molecular consequences of CDK or EBV kinase action on condensin subunits are currently unknown.

Purpose of the study: We aim to determine what are the molecular and cellular effects of phosphorylating condensin on CDK sites during the process of chromosome condensation.

Methods: We use S. cerevisiae as model for this study since this organism possesses only one condensin complex. Standard biochemical approaches have been used to purify the condensin complex and phosphorylate it in vitro using purified CDK kinase. Genetic approaches have been used to create non-phosphorylable and phospho-mimetic mutants of Smc4, the main subunit of the condensin complex targeted by CDK. We monitored DNA condensation using Fluorescence In Situ Hybridization (FISH) in different mutant background to confirm the importance of phosphorylation.

Results: We discovered by mass spectrometry that Smc4 is phosphorylated on 5 CDK consensus sites during mitosis. Furthermore, we show both in vitro and in vivo that Smc4 is a specific substrate for CDK1. Interestingly, we reveal that this modification occurs in early S phase. FISH analysis of yeasts expressing non-phosphorylable or phospho-mimetic forms of Smc4 indicates that these cells display strong condensation defects. We show that chromosome morphology responds quantitatively to modulation of Cdk1 activity, and that a novel chromatin-folding intermediate can be established in the absence of all other mitotic events.

Conclusion: Collectively, our results indicate that the phosphorylation of Smc4 on CDK sites is a crucial regulatory step for the activation of chromosome condensation. We also demonstrate that the dynamic of phosphorylation of Smc4 by CDK is the key element that activates condensin in early mitosis. As a consequence, we hypothesize that preventing the phosphorylation of condensin by the EBV kinase represent a novel and promising therapeutic avenue to treat and even prevent the development of childhood leukemia and lymphoma.

PO16

Title: Ezh2 is an essential regulator of T-cell development and oncogenic transformation in mouse and human adult T cell acute leukemia

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Keywords: T-ALL, EZH2, PRC2, Leukemia, Epigenetic.

Background information: Enhancer of zeste homolog 2 (EZH2) catalyzes di- and trimethylation of lysine 27 on histone H3 (H3K27me2/3) and establishes chromatin marks associated with gene silencing. The enzymatic activity of EZH2 depends on formation of PRC2 complex comprising EZH2, EED and SUZ12. EZH2 mutations representing loss-of-function alleles have recently been identified in myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). Conversely, over-expression of Ezh2 has also been implicated in progression of various types of human cancers, and a recurrent EZH2 mutation identified in B-cell lymphomas was proposed to act as a dominant, cancer-promoting EZH2 allele. Genetic data therefore suggest that gene dosage could be deterministic for the apparently contradictory oncogenic and tumor suppressing activities of EZH2, but no functional data supporting these possibilities have so far been presented.

Purpose of the study: This study aim to better characterize the role of Ezh2 in mouse and human cancer development and more particularly in T-cell acute lymphoblastic leukemia (T-ALL).

Methods: We previously showed that heterozygosity for mutant Eed alleles accelerates lymphomagenesis in mice. For functional study we exploited an Ezh2 conditional knockout mouse model. The Cre-mediated deletion generates a mutated Ezh2Δ allele and abrogates production of EZH2 protein specifically in adult bone marrow cells. Upon gene inactivation we monitored cancer development and characterized leukemic cells with surface markers and proliferation status. We also evaluated histone methylation status as well as PRC2 integrity. To address the integrity of PRC2 and associated genes in adult human T-ALL, we sequenced transcriptomes and exomes of 12 human adult T-ALL specimens using the Illumina HiSeq2000 platform. We analyzed them for nonsynonymous mutations, large and small insertions and/or deletions in PRC2 and other associated genes.

Results: Using next generation sequencing, we identified alteration in gene expression levels of EZH2 and acquired mutations in PRC2-associated genes (DNMT3A, JARID2) in human adult T-ALL. We observed high frequency of spontaneous ∘ ⌒T cell leukemia (T-ALL) occurrence in mice with bi-allelic deletion of Ezh2. Interestingly, lymphoblasts were exclusively positive for the cell surface TCR ∘ ⌒. Cell cycle analysis on pre-leukemic mice revealed an activation of the G2/M checkpoint. Moreover we found that the Ezh2 deficient TCR ∘ ⌒ leukemia were prone to genomic instability. Indeed, the majority of the leukemias were aneuploid and 50% of them were near-tetraploid. Finally, bioinformatics analysis of transcriptomic data from various samples revealed that the genes having the highest correlation factor with Ezh2 are involved in cell division, DNA replication and DNA damage repair.

Conclusion: Our studies provide the first in vivo validation of the proposed tumor suppressive activity of Ezh2 in mice. These results are in line with our previous findings indicating that a loss of Eed function sensitises mice for development of T- and B-ALL. Moreover, the high incidence of other PRC2 genetic alterations observed in human T-ALL, argue for a more generalized deregulation of this Polycomb protein complex and associated genes in T-ALL. Together, these study shows that Ezh2 is an essential regulator of the TCRγδ T-cell state, and prevents T-cell transformation, likely through regulation of DNA replication, cell division or DNA damage repair.

PO17

Title: Inhibition of histone deacetylation as a novel antifungal therapy in leukemia patients

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Keywords: antifungal chemotherapy, leukemia, H3K56 acetylation, Hst3 deacetylase, chemical screen

Background information: Invasive fungal infections are an important cause of morbid ity and mortality for children and adults with acute myeloid leukemia (AML). These patients are at high risk of infection, likely because they receive high doses of chemotherapeutic agents that result in prolonged and severe neutropenia. Current antifungal therapy is limited by the emergence of multidrug resistant fungi or adverse effects such as high toxicity; therefore there is an urgent need for new antifungal strategies to overcome these limitations. Histone H3 lysine 56 acetylation (H3K56ac) and deacetylation play important roles in chromosome assembly during DNA replication and DNA repair in fungi. We previously demonstrated that genetic inactivation or pharmacological inhibition by nicotinamide (NAM) of the fungal-specific histone deacetylase Hst3 both result in H3K56 hyperacetylation and lethality in Candida albicans, which is the most frequently encountered human fungal pathogen. This indicates that Hst3-mediated H3K56 deacetylation represents a promising target pathway for discovery of novel antifungal drugs.

Purpose of the study: Our goal is to discover small molecules that synergize with NAM to inhibit the H3K56 deacetylation pathway and result in high fungicidal activity in C. albicans.

Methods: We performed a high-throughput, cell-based phenotypic screen of a library of 678 compounds preselected for bioactivity against yeast in the presence or absence of NAM. The growth of C. albicans in 96-well assay plates containing test chemicals was measured every 15 minutes for 48 hours. Small molecules that enhance NAM-mediated growth inhibition of C. albicans are considered as primary hits.

Results: From our chemical screen, we identified one compound (CD08396) that, when used alone, causes mild growth inhibition of C. albicans. In striking contrast, combination of CD08396 and NAM dramatically delays the growth of C. albicans by at least 30 hours compared with cell growth in the absence of either chemical or by at least 15 hours compared with the growth of cells treated only with CD08396.

Conclusion: From a preselected chemical library containing yeast bioactive compounds we identified one compound that reproducibly causes growth retardation of C. albicans in the presence of NAM. These compounds are not toxic to humans at the concentrations used in the screen. This suggests that compound CD08396 affects the Hst3-mediated deacetylation pathway either directly or indirectly. We are currently trying to validate this result and identify the cellular target(s) of this compound.

PO18

Title: Self-renewal network activated by SCL-LMO1 confer aberrant stem cell properties to immature thymocytes

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Keywords: self-renewal, SCL, LMO, T-ALL.

Background information: The SCL and LMO1 oncogenes are commonly activated in T-cell acute lymphoblastic leukemia (T-ALL), and these oncogenes collaborate to induce the disease in transgenic mice. Recently, we found that SCL-LMO1 reprogram immature DN3 thymocytes into pre-leukemic stem cells (pre-LSCs) with aberrant self-renewal capabilities. Remarkably, we showed that oncogenic reprogramming is a limiting event in leukemogenesis. We now propose that these self-renewing pre-LSCs provide a reservoir of cells that can acquire additional genetic events and undergo clonal expansion, leading to overt leukemia. In this context, investigating the molecular networks controlling self-renewal in SCL-LMO1 pre-LSCs may provide novel insights on leukemia initiation.

Purpose of the study: Here, our goal was to characterize the self-renewal network activated in pre-LSCs due to oncogenic reprogramming by SCL-LMO1, and to define at the molecular level, the genetic events that provide additional fitness traits driving leukemia progression.

Methods: We performed RNA-seq and microarray experiments to measure the transcriptome of pre-leukemic (pre-LSCs), as well as early and late stage leukemias obtained in the SCL-LMO1 transgenic mouse model of T-ALL. To explore this dataset, we developed a novel systems biology approach that takes advantage of the commonalities between normal and pre-LSCs to find transcription factors (TFs) and targets implicated in the acquisition of self-renewal in DN3 thymocytes.

Results: Our method constructs a self-renewal regulatory network by searching for stem cell TFs that are activated by SCL-LMO1 in DN3 thymocytes, and then connects these regulators to their downstream targets using ChIP-seq data. The network analysis predicted that Lyl1 is a major regulator downstream of SCL-LMO1, and revealed that Scl, Lmo1 and Lyl1 redundantly co-regulate a self-renewal program in DN3 thymocytes. We confirmed by ChIP and reporter assays that SCL-LMO1 binds to Lyl1 promoter, leading to transcriptional activation. We also showed by transplantation assays that LYL1 mimic the effects of SCL to collaborate with LMO1 and induce DN3 thymocyte self-renewal. Additionally, we found by RNA-seq that the SCL-LMO1 self-renewal network is predominantly activated in the early stages of the disease, and is diluted in the bulk of leukemic cells in late stage thymomas, which are marked by an activation of cell cycle and DNA repair genes.

Conclusion: Altogether, these results indicate that the acquisition of self-renewal represents an initiating event in leukemogenesis while disease progression is associated with additional fitness traits such as cell proliferation and survival. These observations have implications for novel therapeutic strategies specifically targeting pre-LSCs self-renewal in T-ALL. Our methodology has shown to effectively predict novel regulators of self-renewal in DN3 progenitors and can be applied to the study of self-renewal networks induced by other oncogenes.

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PO19

Title: The sonic hedgehog factor Gli1 imparts drug resistance through inducible glucuronidation

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Keywords: Gli1, GDC-0449, UGT1A, ribavirin, eIF4E, drug resistance, glucuronidation
Background: Despite many recent successes in the treatment of cancer, the development of chemoresistance in many of the initially responding patients, and primary resistance in others, remains a major impediment in therapy development. Our studies provide evidence for a novel mechanism underlying drug resistance: Gli1 dependent drug glucuronidation.

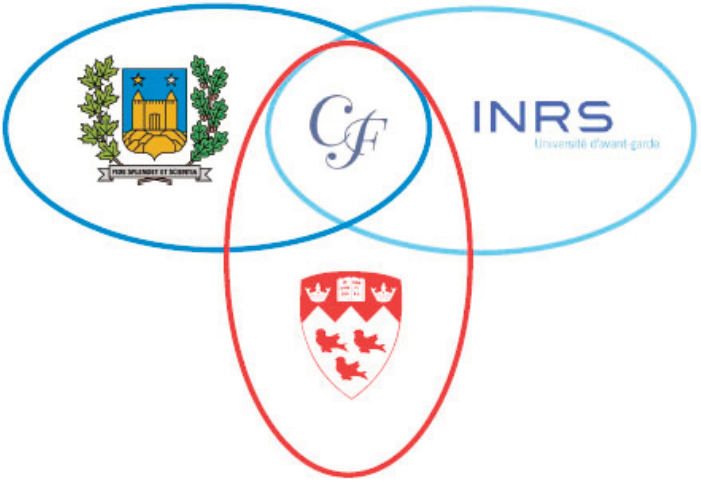
Purpose of the study: While carrying out a Phase II clinical trial of targeting the eukaryotic translation initiation factor eIF4E with ribavirin in M4/M5 subtypes of AML, we observed that all responding patients eventually became clinically and molecularly resistant. To understand the cause of this resistance, we generated ribavirin resistant cell lines. In these models, ribavirin no longer targeted eIF4E activity or impaired growth, and importantly, the ability of ribavirin to bind eIF4E was severely impaired. However, the eIF4E gene was not mutated and its protein levels were not altered. The cell lines could be divided into two groups: type I with a defect in drug uptake and type II with a normal uptake. In type I resistant cells, we observed a substantial reduction in levels of Adenosine Kinase (ADK) an enzyme that catalyzes the rate limiting step in the metabolic activation of ribavirin allowing its retention in the cells.

Methods: We used RNA Sequencing to examine the molecular underpinnings of type II resistance. Our data revealed a drastic increase in the levels of Gli1. In stably overexpressing cells, Gli1 was sufficient to produce the same resistance phenotype that we observed for type II cell models, both molecularly and at the level of cell growth. In addition, Gli1 overexpression correlated with the loss of drug-to-target interaction, as observed by our eIF4E immunoprecipitation studies using 3H-Ribavirin, similarly to the resistant cell lines. Conversely, Gli1 knockdown in type II cells or its pharmacological inhibition with the FDA approved Gli1 inhibitor GDC0449/Vismodegib, restored the eIF4E-ribavirin interaction and re-sensitized these cells to ribavirin.

Results: Our subsequent studies revealed a close correlation between Gli1 expression and the protein levels of the UGT1A glucuronosyl transferase enzymes involved in phase II drug metabolism whereby xenobiotics or metabolites are modified by the addition of a sugar, glucuronic acid. Given these findings, we examined whether the loss of the eIF4E interaction in resistant cells was due to the glucuronidation of ribavirin. Using 13C/12C ribavirin and mass spectrometry, we observed glucuronidated forms of ribavirin in resistant cells and cells overexpressing Gli1 but not in parental cells and that ribavirin is glucuronidated on its triazole ring which binds eIF4E. Treatment of cells with the Gli1 inhibitor GDC0449 reduces UGT1A levels, and correlates with reduced levels of ribavirin-glucuronides and the re-emergence of ribavirin-eIF4E complexes.
We further assessed if type II resistant cells could be resistant to other drugs. We observe that our ribavirin resistant cell lines are also resistant to the cornerstone of AML therapy, cytarabine. GDC0449/Vismodegib treatment reverts resistance to cytarabine in these cells. Preliminary studies indicate that these cells are also resistant to azacytidine and cisplatin. This is particularly striking as these cells were never exposed to these compounds. Thus, this could represent a novel form of multi-drug resistance.
To establish the clinical relevance of our findings to patients in our AML ribavirin trial, we examined features of type I and type II resistance. Out of 10 patient samples available for evaluation, all six responding patient specimens showed elevated Gli-1 mRNA levels, up to 26 fold, upon relapse relative to levels during response. For most, the ratio of Gli1 during response relative to at relapse was about 2-4 fold with some patients up to 10 fold. For the two patients examined that did not respond, both had highly elevated Gli-1 levels prior to treatment relative to healthy individuals, and this was not lowered after 28 days of ribavirin treatment. We also noted elevated UGT1A protein levels upon relapse in our patient population. Type I resistance was observed in only two patients whereas Gli1 and UGT1A were dysregulated at relapse in all patients examined.

Conclusion: In summary, we identified a novel form of drug resistance: Gli1 dependent drug glucuronidation. Treatment with Gli1 inhibitors appears to be a promising avenue for overcoming this form of drug resistance.

Notes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