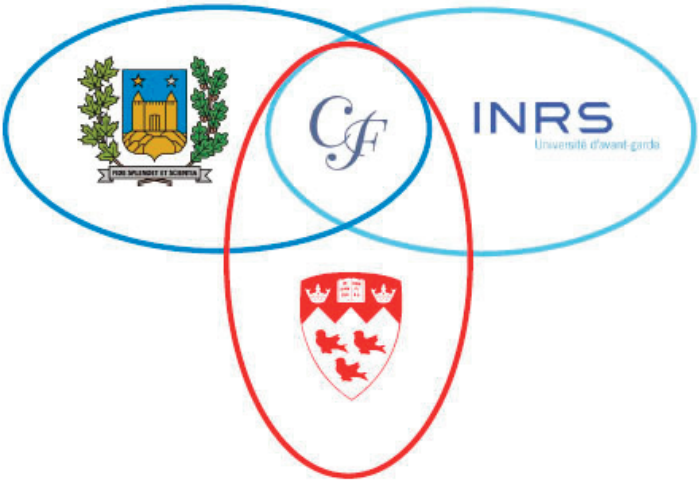
Journée
« Célébrons la recherche »
de la Fondation Cole
10 mai 2013



Cole Foundation
" Research Celebration Day "
May 10, 2013

The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD and MSc 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 2013 Fellows, the Fellowship programme has supported more than 100 researchers in laboratories and hospitals situated in the Greater Montreal area through collaborations with INRS - Institut Armand-Frappier Research Centre; l'Université de Montréal; and McGill University. 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 et de maîtrise ès sciences. Avec l'annonce des boursiers 2013, le programme a appuyé plus de 100 chercheurs répartis dans des laboratoires et des hôpitaux de l'agglomération montréalaise, à la faveur de collaborations avec le Centre INRS – Institut Armand-Frappier, l'Université de Montréal et l'Université McGill. Plus 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 son décès 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.

Programme/Program

9:30 AM - 10:00 AM	Table ronde (nouveaux boursiers) Round Table (new fellows) *Pavillon Roger-Gaudry, salle M-425* *Roger-Gaudry Building, room M-425*
10:00 AM - 11:00 AM	Session d'affichage première partie Poster Session Part 1
11:00 AM - 12:00 PM	Session d'affichage première partie Poster Session Part 2
12:00 PM - 1:00 PM	Dîner / Lunch
1:00 PM - 2:00 PM	Nouvelles idées - nouvelle génération : Chercheurs de la Fondation Cole New Ideas - New Generation: Cole Foundation Investigators
2:00 PM - 3:15 PM	Conférence / Lecture *Amphithéâtre M-415* *Amphitheater M-415*
Bienvenue / Welcome	*Barry Cole* *Président / President* *Cole Foundation*
Introduction / Introduction	*Daniel Sinnett* *Professeur, département de pédiatrie, Université de Montréal* *Professor, pediatric department, Université de Montréal*
Conférencier invité / Keynote Speaker	*Dr David Malkin* *Senior Staff Oncologist, Hematology/Oncology, Director Cancer Genetics Program, SickKids Hospital, Toronto*
Allocution du recteur / Rector's Speech	*Dr Guy Breton*
3:15 PM - 3:30 PM	Nouveaux boursiers et remise des prix New fellows and announcement of prizes
3:30 PM	Réception / Reception

Les boursiers de recherche de la Fondation Cole 2013 - 2015
The 2013 - 2015 Cole Foundation Fellows

Université de Montréal

Magalie Celton, Post PhD program
Supervisor: Brian Wilhelm, IRIC, Médecine
Project Title: Identification and investigation of the collaborating molecular events in pediatric acute myeloid leukemia with MLL-AF9 translocation
Description: This project involves identifying molecular events that collaborate with the MLL-AF9 translocation to cause pediatric acute myeloid leukemia. In addition, this project will investigate the role of these collaborating events in the initiation and evolution of leukemia.

Bastien Gerby, Post PhD program
Supervisor: Trang Hoang, IRIC, Hematopoiesis & Leukemia Research
Project Title: Towards targeted therapy of T-acute lymphoblastic leukemia
Description: My project aims to develop a mechanism-based therapy targeting the abnormal stem cell properties induced by oncogenes in pre-Leukemic Stem Cells. This project should help to develop active and specific drug targeting the aberrant functions of oncogenes involved in initiation, maintenance and deve lop-ment of paediatric acute lymphoblastic leukemia.

Damien Grapton, PhD program
Supervisor: Tarik Möröy, IRCM, Experimental Medicine
Project Title: Étude de rôle de la forme variante de GFI1 (GFI136N) dans le développement de la leucémie aiguë myéloblastique
Description: A variant form of a small nuclear protein called Gfi1 for “Growth Factor Independent 1” is associated with increased risk of developing Acute Myeloid Leukemia (AML). We try to understand the molecular mechanisms that are triggered by Gfi1 in this regard.

Jean-Philippe Guégan, Post PhD program
Supervisor: Sylvain Meloche, IRIC, Pharmacologie
Project Title: Rôles de TC21 et de la voie MAPK dans le développement et la progression des leucémies myéloïdes
Description: La protéine TC21 présente un rôle pro-oncogénique dans dif-férentes tumeurs et est surexprimée dans les cellules leucémiques agressives. Néanmoins son implication dans la leucémogénèse n'a pas été étudiée. Notre projet vise donc à tester le rôle de TC21 dans la tumorigénèse et l'importance de sa régulation par la voie MAPK.

Elizabeth Krakow, Clinician
Supervisor: Jean-Sébastien Delisle, Hôpital Maisonneuve-Rosemont, Hématologie-Oncologie
Project Title: Adoptive Transfer of Alloreactive Cells to Treat Poor-Prognosis Acute Myeloid Leukemia (ATAC-AML-01)
Description: Nous infusons des cellules immunitaires HLA-incompatibles provenant d'un donneur sain après une chimiothérapie chez des patients souffrant de leucémie myéloïde aiguë en rechute ou résistants à la chimiothérapie et inéligibles à l'allogreffe. Le but est d'amener une réponse anti-leucémique, avant que les cellules du donneurs soient rejetées par le patient, permettant ainsi d'éviter des effets secondaires importants tout en espérant une activité anti-leucémique.

Arhamatoulaye Maiga, Post PhD program
Supervisor: Michel Bouvier, IRIC, Biochimie
Project Title: Récepteurs couplés aux protéines G (RCPG); Leucemie Aigue Myeloide (LAM); Criblage chimique: cible therapeutique; Transcriptome
Description: Ce projet s'intéresse aux récepteurs couplés aux protéines G (RCPG), une famille de protéines à fort potentiel thérapeutique, en vue de concevoir de nouvelles molécules plus spécifiques pour le traitement des leucémies myéloïdes aigues (LMA).

Camille Simon, PhD program
Supervisor: Guy Sauvageau, IRIC, Médecine
Project Title: The Polycomb Repressive omplex 2 (RPC2): a mechanistic investigation of its role in normal and leukemic stem cells
Description: The polycomb repressive complex 2 (PRC2) consists of a number of proteins that together regulate the activity of stem cells in our body My research project aim to study the role and molecular regulation of key components of the PRC2 complex in hematopoietic stem cells and leukemia.

Hiba Zahreddine, PhD program
Supervisor: Katherine Borden, IRIC, Pathology and Cell Biology
Project Title: Understanding Clinical Resistance to Ribavirin in M4/M5 Acute Myeloid Leukemia patients
Description: My research project aims at understanding the molecular mechanism(s) underlying one of the cancer resistance cases observed in patients with AML treated with a drug called ribavirin. Based on my preliminary results we hypothesized that Gli1 drives resistance by altering gene expression of UGT1A enzymes resulting in enhanced drug metabolism.

McGill University

Alicia Bolt, Post PhD program
Supervisor: Koren Mann, Lady Davis Institute, Experimental Medicine/Oncology
Project Title: The role of tungsten in preB acute lymphoblastic leukemogenesis
Description: High levels of tungsten have been observed near three pediatric leukemia clusters, yet no causative link exists. My project will investigate the mechanisms of tungsten-altered B cell development and determine whether tungsten can provide one of the two hits required for the development of leukemia using a mouse model.

Julie Ross, Post PhD program
Supervisor: Tarik Möröy, IRCM, Experimental Medicine
Project Title: Role of the BTB/POZ domain protein Miz-1 in the development of B-cell lymphoma
Description: Abnormal expression of the oncoproteins c-Myc or Bcl6 is frequently found in B-cell lymphomas affecting children and young adults. Miz-1 is a partner of c-Myc and Bcl6. Using different mouse models, we will study the role of Miz-1 in the development of normal and malignant B-cell.

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

Liste des exposants / Presenters

Aissiou, Mohamed
Ashton-Beaucage, Dariel
Assaker, Gloria
Bisaillon, Richard
Davoudi, Sayeh
Dupéré-Richer, Daphne
Fang, Yi
Gauthier, Simon-David
Gerby, Bastien
Hariri, Fadi
Lisi, Veronique
Mancini, Johanna
Methot, Stephen
Montpas, Nicolas
Oussa, Eustache
Pabst,Caroline
Pécheux, Lucie
Rashkovan, Marissa
Robellet, Xavier
Siddiqui, Nadeem
Simon, Camille
Spinella, Jean-Francois
Tan-Ning Tsao, Sarah
Vazquez, Gabriela
Veiga, Diogo

Liste des projets exposés / Poster List

No.	Name	Title
PO1	Aissiou, Mohamed	Early detection of doxorubicin-induced cardiotoxicity using multiparametric MRI and biomechanical modeling: Proposed model for childhood leukemia
PO2	Ashton-Beaucage, Dariel	Identification of factors controlling mapk stability by rnai-based synthetic genetic interaction screening
PO3	Assaker, Gloria	Regulation of Delta ligand activity in Notch signaling
PO4	Bisaillon, Richard	Identification of MEIS-PBX inhibitors as potential anti-leukemic agents using a high-throughput BRET-based assay
PO5	Davoudi, Sayeh (absente)	The Role of Translesion Synthesis Polymerase REV1 in the Generation of Point Mutations in Mammalian Cells

PO6	Dupéré-Richer, Daphne	Vorinostat-induced autophagy switches from a death promoting to a cytoprotective signal to drive acquired resistance
PO7	Fang, Yi	Vesicular communication between leukemic cells and vascular stroma – transfer of signaling and oncogenic molecules from acute myeloid leukemia to endothelial cells
PO8	Gauthier, Simon-David	Flt3-ligand and IL-7 treatments can restore homeostatic proliferation of naive CD4+ T cells during acute GVHD
P09	Gerby, Bastien	The SCL, LMO1 and Notch1 oncogenes reprogram T-lymphocyte progenitors into self-renewing pre-leukemic stem cells
PO10	Hariri, Fadi	The eukaryotic translation initiation factor eIF4E is a direct transcriptional target of NF-κB and is aberrantly regulated in Acute Myeloid Leukemia
PO11	Lisi, Veronique	Understanding the microRNAs regulatory network through computational modeling and molecular biology
PO12	Mancini, Johanna	Regulation and targeting of telomere maintenance
PO13	Methot, Stephen	EEF1A1 is part of the AID cytoplasmic retention complex, which is in dynamic equilibrium with Hsp90
PO14	Montpas, Nicolas	Potential role of CXCR7 as a negative modulator of leukemia bone marrow niche homing

PO15	Oussa, Eustache	Importance of TRAF1 phosphorylation on the recruitment of TBK1 in CD8 T cells
PO16	Pabst, Caroline	Aryl-hydrocarbon receptor activation induces differentiation of primary myeloid leukemia stem/progenitor cells
PO17	Pécheux, Lucie	Modifications of histone methylation can modulate hematopoietic stem cell fate decisions
PO18	Rashkovan, Marissa	Role of Miz-1 (Myc-Interacting Zinc-finger protein-1) as a regulator of p53 dependent pathways during pre-TCR selection at the DN3 β selection checkpoint
PO19	Robellet, Xavier	A Cdk1-Dependent Phospho-Switch to Initiate Mitotic Chromosome Condensation
PO20	Siddiqui, Nadeem (absent)	Elucidating the structure of the catalytic ATP binding domain from human mTOR
PO21	Simon, Camille	Ezh2 is an essential regulator of T-cell development and oncogenic transformation in mouse and human T cell acute leukemia
PO22	Spinella, Jean-Francois (absent)	Ras-MAPK signaling and chromatin remodeling are key driver pathways in childhood pre-B acute lymphoblastic leukemia
PO23	Tan-Ning Tsao, Sarah	Inhibition of histone deacetylation as a novel antifungal therapy in leukemia patients

PO24	Vazquez, Gabriela	Suppression of Eukaryotic Initiation Factor 4A Chemosensitizes Myc driven lymphomas to ABT-737 therapy
PO25	Veiga, Diogo	Integrative approach to discover mo lecular events transforming pre-leukemic thymocytes into leukemic T-ALL cells

PO1

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

Author: Mohamed Aissiou

Affiliation: Dr Delphine Périé-Curnier, Mehcanical Engineering Department, École Polytechnique de Montréal

Keywords: doxorubicin cardiotoxicity, multiparametric MRI, 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. Using muscle fibers orientation (e.g., DT-MRI) or 3D imaging techniques (e.g., Cine-DENSE MRI) are a promising alternatives, however, their clinical application is limited due to the acquisition time and their estimation errors. In parallel, the use of multiparametric MRI (T1, T2 and T2*) and finite-element modeling of the myocardium has demonstrated great potential for early detecting doxorubicin-induced cardiomyopathies.

Purpose of the study: Propose an imaging-based method to quantify myocardial biochemical and mechanical characteristics using multiparametric and Cine MRI. The hypothesis is that doxorubicin chemotherapy induces direct damage to myocardial fibers architecture which can be detected using both biomechanical modeling and multiparametric MR parameters analysis.

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. T1, T2 and T2* sequences were acquired at the mid-ventricular level in short axis using the same spatial resolution. Once mapped, the mean T1, T2 and T2* relaxation times was measured within the ventricular septum. The overall acquisition was approximately 25 minutes. To extract myocardial deformation, myocardial wall contours were extracted and tracked at each phase and parameterized using per-length normalization under Matlab (The MathWorks, Inc.). The 3D displacement at each point was assembled using component-by-component assembly and injected to the reconstructed model of the ventricles using Catia V5 (Dassault Systèmes, Inc.). Displacement errors were estimated between the reconstructed model (reference) at mid-systole (MS=200ms) and the simulated model, obtained by adding the generated 3D displacements to the reconstructed end-diastolic model (ED=0ms).

Results: Estimated Root-Mean-Square (RMS) errors between MS and simulated MS (ED + generated 3D displacements) geometries was 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. The mean T1, T2 and T2* relaxation times were 1029±90 ms, 56±4 ms and 38±5 ms, respectively.

Conclusion: Deformation patterns analysis successfully allowed a personalized reconstruction and tracking of the 3D myocardial deformation. RMS errors of estimated displacements were relatively high and overestimated because of the gross discretization of the geometry. We believe that the use of future refined mesh would significantly improve displacements estimation. The use of multiparametric MRI demonstrated great feasibility and an acquisition time suited for a future clinical application. However, analysis may be limited to the ventricular septum to reduce induced noise due to mapping.

PO2

Title: Identification of Factors Controlling MAPK Stability by RNAi-Based Synthetic Genetic Interaction Screening

Author: Dariel Ashton-Beaucage

Affiliation: Marc Therrien, Département de pathologie et biologie cellulaire, IRIC, Université de Montréal

Keywords: RAS oncogene, MAPK signalling, ubiquitination, drosophila

Background information: The small GTPase RAS is the most prevalent human oncogene. The evolutionarily conserved RAF-MEK-MAPK signaling cassette that lies downstream of RAS is the main conduit through which RAS transmits both physiological and tumorigenic signals. To date, many factors that act to modulate pathway component activity have been identified. However, comparatively little is known on how the expression and turnover of core RAS/MAPK factors is controlled.

Purpose of the study: We seek to improve our understanding of how MAPK – a central component of the RAS/MAPK pathway – is controlled.

Methods: Recently, we identified the deubiquitinating enzyme (DUB) Ubp64E in a genome-wide RNAi screen for factors acting downstream of RAS in cultured Drosophila cells. Subsequent screening assays positioned the impact of Ubp64E RNAi downstream of MEK and revealed that it caused a decrease in MAPK protein levels. In order to identify potential E2 and E3 enzymes that might be counteracting the effect of this DUB, we conducted a targeted screen to identify factors that displayed a synthetic alleviating effect when co-depleted with Ubp64E.

Results: Using this strategy, we were successful in identifying components of the general ubiquitin/proteasome machinery as well as one E2 (UbcD6) and two E3 (poe and CG5604) enzymes that could rescue the effect of Ubp64E RNAi on MAPK levels. These three factors have been linked to a degradation mechanism termed “N-end rule” that proceeds through recognition of a degron located in the N-terminal end of the protein. This raises the possibility that MAPK turnover is controlled through N-end rule.

Conclusion: Our findings demonstrate that synthetic genetic screening by RNAi is an effective approach to identify functionally related factors. Moreover, our screening results suggest that regulation of MAPK degradation is an important and previously unappreciated layer of this signaling network. Specifically, Ubp64E and UbcD6/poe/CG5604 seem to play opposite regulatory roles in the control of MAPK turnover. The role of these factors may extend beyond the context of RAS signalling to other targets that possess features in common with MAPK.

PO3

Title: Regulation of Delta ligand activity in Notch signaling

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, screen, T-ALL.

Background information: The signaling receptor Notch plays a key role in stem cell self-renewal, cell proliferation, differentiation, and apoptosis. Consequently, it is involved in many diseases and cancers. Understanding how Notch signaling is regulated is thus central to understand cancer development and to identify new potential therapeutic targets. The Notch receptor responds to the transmembrane ligand Delta and many cancers involving Notch are ligand-dependent. For instance, activating mutations in NOTCH1 have been identified in over 50% of T-cell Acute Lymphoblastic Leukemias (T-ALL), with 40% of these mutations resulting in ligand hypersensitivity or ligand-independent Notch activation. It has been shown that the Notch ligand Delta needs to be ubiquitinated and endocytosed to transactivate Notch. However, the molecular mechanisms regulating Delta activation are still poorly characterized.

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

Methods: To unravel new regulators of Delta, we performed a genome-wide shRNA screen using an in vitro co-culture assay. In this assay, we used the OP9-DL1 cell line as a signal sending cell. OP9-DL1 cells are bone marrow stromal cells that have been stably transduced to express one of the mouse homologs of Delta (DL1). As signal-receiving cells, we used HeLa cells that natively express the Notch receptor (NOTCH1), and that we stably transfected with a luciferase reporter of Notch activity. We are further developing functional secondary assays using the OP9-DL1 model in order to validate our primary hits in physio-pathological contexts. We will first investigate whether the identified genes are involved in Notch mediated events, such as the differentiation of hematopoietic progenitors into T-cells. Then we will test whether they are required for the maintenance of primary leukemic T-ALL cells in vitro, and for leukemia development in vivo using transplantation experiments in mice**.**

Results: The primary shRNA screen that we performed led to the identification of 665 hits as positive regulators of the Notch ligand Delta. We were able to validate 166 genes out of these primary hits through reconfirmation screens where we repeated the primary luciferase assay on our hits to verify their phenotype. Interestingly, among the validated hits that came out of our screen, we found genes that are known to be involved in the pathogenesis of T-ALL.

Conclusion: This study allowed us to unravel new regulators of Notch signaling, with possible translation into the clinic for novel anti-Notch therapies in cancer, especially in T-ALL. 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. This project therefore represents a unique opportunity to identify new hits as potential molecular therapeutic targets for the treatment of T-ALL.

PO4

Title: Identification of MEIS-PBX inhibitors as potential anti-leukemic agents using a high-throughput BRET-based assay

Author: Richard Bisaillon, Eva Schmidt, Anne-Sophie Guenier

Affiliation: Dr Guy Sauvageau, Department of Molecular Biology, Institute for Research in Immunology and Cancer (IRIC), University of Montreal

Keywords: Acute myeloid leukemia, Hox cofactors, BRET

Background information: The deregulated expression of HOX genes and their MEIS1/PBX co-factors represents one of the most frequent molecular anomalies in human acute myeloid leukemia (AML), most prominently in those with chromosomal translocation in the MLL gene. In mouse genetic models, over-expression of MEIS1 significantly accelerates the onset of HOX-induced AML, a unique function that requires heterodimerization with its PBX cofactor. Importantly the expression of MEIS1 is critical for MLL-fusion-induced leukemogenesis, suggesting that MEIS1 inhibition represents a good therapeutic target for AML treatment.

Purpose of the study: We propose first to identify chemical compounds that disrupt the MEIS1-PBX1 interaction using a Bioluminescence Resonance Energy Transfer (BRET)-based high-throughput assay and second, to test the effective compounds for anti-leukemic activity.

Methods: The BRET technique monitors protein-protein interaction by employing energy transfer from a bioluminescent enzyme (e.g. Luciferase) to a fluorescent protein (e.g. GFP) located in very close proximity, yielding an interaction-specific emission light signal. The fusion constructs LUC2MEIS1 and GFP10PBX1 are transfected transiently in HEK293T cells and the luminescence (LUC) and fluorescence (GFP) activity is monitored following addition of the Luciferase substrate Coelenterazine 400a. The BRET signal originating from a PBX-interaction-inapt Meis1 mutant (LUC2MEIS1àPIM) serves as negative control.

Results: The transient transfection of [LUC2MEIS1 + GFP10PBX1] resulted in a BRET signal of 0.35±0.02 (A.U.) following substrate addition whereas [LUC2MEIS1àPIM + GFP10PBX1] resulted in a BRET signal of 0.170±0.007 (A.U.). The assay, with a Z-factor of 0.81, is robust enough for high-throughput screening and the library of 100,000 compounds available at IRIC is currently being tested. A pilot “run” already processed 2880 compounds, of which 11 reduced the BRET ratio without perturbing/quenching the individual LUC or GFP signal. The potential inhibitors will be further tested for their ability to prevent dimerization of MEIS1-PBX1 using a modified Luciferase ImmunoPrecipitation System (LIPS) assay. The validated hits will then go through a secondary screen monitoring cell proliferation of human AML samples expressing various levels of HOX/MEIS/PBX.

Conclusion: If successful this screen will provide us with multiple molecular tools to mo dulate the activity of the MEIS-PBX complex. Given the requirement of this complex for transformation induced by numerous oncogenes, these tools could eventually lead to mechanistic-based preclinical studies for personalized therapeutic interventions.

PO5 *(absente / not present)*

Title: The Role of Translesion Synthesis Polymerase REV1 in the Generation of Point Mutations in Mammalian Cells

Author: Sayeh Davoudi

Affiliation: Dr. Alain Nepveu, Goodman Cancer Research Centre, McGill University

Keywords: chronic myelogenous leukemia, BCR/ABL, Imatinib mesylate, resistance

Background: Chronic myelogenous leukemia (CML) is caused by a chromosomal translocation that fuses the BCR and ABL genes. Imatinib mesylate (IM), a drug that inhibit the BCR-ABL protein, has become the treatment of choice for patients with chronic-phase CML. Despite amazing success, treatment with IM fails in 25% of cases due to primary or secondary resistance. Resistance to imatinib most often results from point mutations in the BCR-ABL catalytic domain that prevent binding of the IM inhibitor. Point mutations are generated by an active process that involves the recruitment to DNA of error-prone DNA polymerases that carry translesion synthesis (TLS). In mammals, the REV1 protein functions as a scaffolding protein that recruits TLS polymerases to DNA. REV1 itself is recruited to DNA via its N-terminal region which also mediates dimerization.

Purpose of the study: The aim of this project was to evaluate whether experimental manipulations that affect the rate of point mutations can also change the frequency of resistance to IM. If confirmed, this notion could lead to a strategy to reduce the resistance to IM.

Methods: We have established a tissue culture system that recapitulates the acquisition of resistance to imatinib by leukemic cells. Briefly, transformation of IL3-dependent BaF3 pre-B lymphocytes with a vector expressing BCR-ABL generated a population of cells that can grow in the absence of IL-3. The addition of imatinib inhibited the BCR-ABL kinase and effectively caused cell death. However, at a low frequency, some resistant clones arose that carry point mutations in the BCR-ABL catalytic domain. These mutations are identical to the mutations found in CML patients that suffer a relapse of the disease. We generated BCR-ABL transformed BaF3 cells that stably express the N-terminal region of the REV1 protein and measured the frequency of resistance to IM.

Results: Populations of cells that stably expressed the REV1 N-terminal fragment exhibited a higher frequency of resistance to IM. This observation was reproduced in several independent populations of BCR-ABL transformed BaF3 cells. Molecular analysis demonstrated that the resistant clones had acquired point mutations in the BCR-ABL coding sequences, as observed during relapse in CML patients. Chromatin immunoprecipitation assays revealed an increase in the recruitment of REV1 to the BCR-ABL gene in cells that stably expressed the REV1 N-terminal fragment.

Conclusions: Using a tissue culture system, we have demonstrated that the frequency of resistance to IM correlates with the ability to recruit REV1 to the BCR-ABL gene. Importantly, the demonstration that some experimental manipulations can modulate the rate of point mutations within BCR-ABL suggest a therapeutic strategy to prevent the resistance to IM. Small molecules that interfere with the recruitment of REV1 to DNA would be able to reduce the frequency of point mutations and prevent the resistance to IM. Once developed, such an inhibitor could be given to CML patients in conjunction to IM in order to prolong the period of remission.

PO6

Title: Vorinostat-induced autophagy switches from a death promoting to a cyto-protective signal to drive acquired resistance

Author: Daphné Dupéré-Richer

Affiliation: Dr. Wilson H. Miller Jr., Lady Davis Institute for Medical Research

Keywords: HDAC inhibitors, autophagy, drug resistance

Background information: Histone deacetylase inhibitors (HDACi) have shown promising activity against hematological malignancies in clinical trials and have led to the approval of vorinostat for the treatment of cutaneous T-cell lymphoma. However, as with many cancer therapies, de novo resistance is common and acquired resistance inevitably follows sensitivity. This issue is particularly difficult to resolve in HDACi therapy, as the mechanism of action is still unclear and may involve several components.

Purpose of study: Our objective was to understand the molecular mechanisms underlying resistance to HDACi in order to design better combination strategies and to identify predictive biomarkers for response to HDACi therapy.

Methods: To gain insight into HDACi resistance, we developed vorinostat resistant clones using a dose escalation protocol from the monocytic-like histiocytic lymphoma cell line U937 and the diffuse large B-cell lymphoma SUDHL6. To begin to define pathways involved in resistance to vorinostat, we evaluated the lethal dose (LD)50 of various drugs with different mechanisms of action in resistant cells versus their parental counterpart.

Results: Interestingly, we found that the resistant cells exhibit increased sensitivity toward chloroquine, an inhibitor of autophagy. Consistent with this, resistant cells growing in vorinostat show increased autophagy. Inhibition of autophagy in vorinostat resistant U937 cells by knockdown of Beclin1 or Lamp-2 restores sensitivity to vorinostat. Interestingly, autophagy is also activated in parental U937 cells by de novo treatment with vorinostat. However, in contrast to the resistant cells, inhibition of autophagy decreases sensitivity to vorinostat. Moreover, inducers of autophagy (such as mTOR inhibitors) synergize with vorinostat to induce cell death in parental cells, while the resistant cells remain insensitive.

Conclusion: These results indicate that autophagy can switch from a pro-apoptotic signal to a pro-survival function driving acquired resistance to vorinostat, and highlight the importance of designing combination strategies using modulators of autophagy and HDACi for the treatment of hematological malignancies.

PO7

Title: Vesicular communication between leukemic cells and vascular stroma – transfer of signaling and oncogenic molecules from acute myeloid leukemia to endothelial 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. In this regard, several forms of acute myeloid leukemia (AML) arise as a result of oncogenic gene fusion events. In acute promyelocytic leukemia (APL), oncoprotein PML-RARa-dependent signals block cellular differentiation leading to upregulation of tissue factor (TF), resulting in disseminated intravascular coagulation (DIC). Targeting PML-RARa involving all-trans retinoic acid (ATRA), triggers cellular differentiation, contributes to disease remission and reverses TF-related coagulopathy. One area that has not been studied in this context is the intercellular trafficking of the oncogenic PML-RARa as cargo of extracellular vesicles (EVs - exosomes and ectosomes) and the consequences of this recently discovered process for interaction of leukemic cells and stroma and effects of targeted therapy with ATRA.

Purpose of the study: In this study, we explored the vesiculation of APL cells to elucidate the role of this process in intercellular communication and transfer of the oncogenic PML-RARa between leukemic and endothelial (niche) cells, and the consequences of this for growth, angiogenesis and coagulation.

Methods: Vesiculation of cultured NB4 cells (APL) containing a PML-RARa oncogene was studied using differential centrifugation and Nanoparticle Tracking Analysis (NTA). Intercellular transfer of EVs to endothelial cells (HUVEC) was interrogated using for fluorescent (PKH26) cell membrane labeling, FACS, molecular analysis (Western, RT-PCR, SAB arrays) and bioassays (MTS, cell migration).

Results: Exposure of NB4 cells to ATRA causes cellular differentiation (CD11b), alteration in the angiogenic profile and changes in cellular vesiculation (increased production of small exosomes with few larger ectosomes). TF is a regulatory target of PML-RARa, and we found that EVs shed from NB4 cells contain bioactive TF protein, as well as transcripts encoding both TF and the PML-RARa oncogene. Endothelial cells exposed to NB4-related EVs acquire the expression of tumor-related TF and PML-RARa. Surprisingly, the exposure of endothelial cells to leukemic EVs inhibits their migratory phenotype. The nature of these effects on endothelial cell coagulant, singaling, differentiation and angiogenic properties are being studied in vitro and in vivo.

Conclusion: Leukemic cells emit EVs containing chimeric oncogenes (oncosomes) and this material is readily taken up by normal endothelial cells. Targeted agents such as ATRA alter vesiculation of leukemic cells, suggesting a role of the PML-RARa oncogenic pathway in this process. In spite of the pro-angiogenic phenotype of untreated APL cells their exosomes inhibit migration of endothelial cells. Thus, leukemic cells may alter their cellular surroundings and cause systemic effect by emitting bioactive cargo contained in exosomes and by directly spreading oncogenic molecules.

PO8

Title: Flt3-ligand and IL-7 treatments can restore homeostatic proliferation of naive CD4+ T cells during acute GVHD.

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Keywords: Leukemia, Bone marrow transplantation, GVHD, CD4 homeostatic proliferation, dendritic cells.

Background information: Allogeneic hematopoietic stem cell transplantation (SCT) is the best treatment for numerous types of hematological malignancies. However, graft-versus-host disease (GVHD) is the major cause of morbidity and mortality and its effect on T cell regeneration greatly exacerbates the immunodeficiency normally associated with this treatment. As a result, patients with GVHD are profoundly lymphopenic and T cell reconstitution can take up to several months or years. Current models hold that T cell receptor (TCR) and interleukin-7 (IL7) work together to maintain T cell numbers in the periphery

Purpose of the study: In this work, we hypothesize that GVHD constrains T cell regeneration by damaging the peripheral niche that regulates CD4 homeostatic proliferation (HP). We previously reported that IL7Rα-/- mice have a peripheral niche that is highly permissive for homeostatic proliferation (HP) of CD4+ T cells. Given that CD4+ T cells regeneration is impaired during GVHD, we hypothesized that regenerating a peripheral niche permissive to CD4 HP using IL7Rα-/- bone marrow (BM) might improve CD4 recovery in GVHD hosts after SCT.

Method: To study the impact of GVHD on the peripheral niche regulating CD4 HP, we used the mouse model B6 into B6D2F1. Since IL7Rα-/- DCs support efficiently HP of naïve CD4+ T cells during lymphopenia, we transplanted BM stem cells from B6IL7Rα-/- mice and induced GVHD by adding 1x106 B6 T cells. Twenty-eight days later, we transferred CFSE labelled anti-HY CD4+ T cells (Marilyn) into GVHD hosts and measured their homeostatic proliferation in order to probe the integrity of the peripheral niche.

Results: In non GVHD hosts, Marilyn T cells underwent robust HP while HP was completely abrogated in GVHD hosts. Absence of HP during GVHD was associated with a severe depletion of all DC subsets. Low number of DCs during GVHD was in part due to their elimination by GVHD T cells but most importantly to a myelosuppression affecting DC production from BM stem cells. Interestingly, treatment of GVHD mice with FLT3 ligand (FL) significantly increased the number of DCs, yet it was insufficient for restoring CD4 HP. Given that IL7 mRNA levels in stromal cells were greatly reduced in GVHD hosts, we provided both FL and IL7 to GVHD mice and as predicted, HP of Marilyn CD4+ T cells was significantly increased in this setting.

Conclusion: Thus far, our data support a model wherein loss of CD4 HP during GVHD relates to lower numbers of DC and diminished systemic IL7. For the first time, we demonstrate that the combination of FL and IL7 could be used to restore CD4 HP in GVHD patients. These results represent a proof of concept that immunocompetence can be restored after allogeneic stem cell transplantation.

PO9

Title: The SCL, LMO1 and Notch1 oncogenes reprogram T-lymphocyte progenitors into self-renewing pre-leukemic stem cells

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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: In this work, 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, in vitro by co-culture and video-microscopy and at the molecular level using micro-array analysis and bio-informatic integrative approaches.

Results: Our results indicate that the SCL and LMO1 collaborate to convert DN3 thymocytes into self-renewing pre-leukemic stem cells (pre-LSCs) through the induction of the functional stem cell genes LYL1 and HHEX. This activity is dependent on the direct physical interaction of SCL and LMO1 proteins, as assessed through the use of an LMO-interaction deficient mutant of SCL. Interestingly, our studies reveal also a network of self-renewal genes in thymocyte transformation, involving SCL, LYL1, LMO1, LMO2 and HHEX. Furthermore, we show that the Notch1 oncogene by itself does not induce self-renewal properties to thymocytes but confers a competitive advantage to SCL-LMO1-induced pre-LSCs by favoring cell divisions, resulting in a dramatic expansion of the pool of self-renewing pre-LSCs.

Conclusion: Therefore, the SCL-LMO1 oncogenic transcription factors reprogram DN3 thymocytes to acquire self-renewal potential, thereby establishing a pre-leukemic state. Finally, 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.

PO10

Title: The eukaryotic translation initiation factor eIF4E is a direct transcriptional target of NF-κB and is aberrantly regulated in Acute Myeloid Leukemia

Authors: Fadi Hariri , Meztli Arguello , Laurent Volpon , Biljana Culjkovic , Torsten Nielsen , John Hiscot, Koren Mann, Katherine Borden.

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Keywords: eIF4E, Acute Myeloid Leukemia, NF-κB

Background Information: The eukaryotic translation initiation factor eIF4E is a potent oncogene elevated in many cancers including the M4 and M5 subtypes of acute myeloid leukemia (AML). While eIF4E RNA levels are elevated 3-10 fold in M4/M5 AML, the molecular underpinnings of this dysregulation were unknown. Our previous findings in M5 AML suggested a tantalizing link between eIF4E and NF-κB.

Purpose of this study: Our goal was to investigate whether eIF4E is a direct transcriptional target of NF-κB that is dysregulated preferentially in M4 and M5 AML.

Methods: We evaluated eIF4E transcript levels in primary hematopoietic cells and cell lines induced with NF-κB activating stimuli (PMA). To verify that our findings were NF-κB specific, we used pharmacological (BAY 11-7082) or genetic inhibition (IκB-SR) of NF-κB. We performed ChIP and gel shift assays to monitor NF-κB recruitment to the eIF4E promoter following PMA stimulation in cell lines. Furthermore, we compared NF-κB recruitment to the eIF4E promoter between different AML subtypes (M1, M2, M4 and M5)

Results: In primary hematopoietic cells and in cell lines, eIF4E levels are induced by NF-κB activating stimuli. Pharmacological or genetic inhibition of NF-κB represses this activation. The endogenous human eIF4E promoter recruits p65 and cRel to evolutionarily conserved κB sites in vitro and in vivo following NF-κB activation. Transcriptional activation is demonstrated by recruitment of p300 to the κB sites and phosphorylated Pol II to the coding region. In primary AML specimens, generally we observe that substantially more NF-κB complexes associate with eIF4E promoter elements in M4 and M5 AML specimens examined than in other subtypes or unstimulated normal primary hematopoietic cells. Consistently, genetic inhibition of NF-κB abrogates eIF4E RNA levels in this same population.

Conclusion and Relevance: We demonstrate that eIF4E is a direct transcriptional target of NF-κB that is dysregulated preferentially in M4 and M5 AML. These findings provide novel insights into the transcriptional control of eIF4E and a novel molecular basis for its dysregulation in at least a subset of M4/M5 AML specimens. These findings suggest that there could be eventual clinical utility in controlling the transcription of eIF4E with the use of NF-κB inhibitors, in addition to directly inhibiting eIF4E activity with ribavirin.

PO11

Title: Understanding the microRNAs regulatory network through computational modeling and molecular biology.

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Keywords: microRNA, transcription factors, modeling, leukemia, miRNA target prediction.

Background: microRNAs (miRNAs) are small (19-24 nts) non-coding RNAs that repress the translation of their target genes by pairing to their messenger RNA (mRNA). Each miRNA can regulate hundreds of genes (1) and most genes are targeted by miRNAs (2). MiRNAs' expression is precisely regulated and their misregulation is associated with various cancers, including leukemia (3). The identification of miRNAs' biologically active targets is a difficult problem, in part because their binding sites are defined by only seven nucleotides, a necessary but not sufficient condition for effective targeting (4).

Purpose of the study: The purpose of this study is to decipher miRNAs' regulatory networks through the identification of their targets and the subsequent annotation of their function.

Methods: To gain insights on the miRNAs' regulatory network, we computationally modeled two specific aspects of miRNAs. We studied the miRNAs' network through the identification of regulatory loops between miRNAs and transcription factors (TFs) using publicly available information. This modelling approach was followed by an experimental validation of selected predicted loops. We used the context of hematopoiesis to address the biological role of these loops. We also studied the physical interaction between miRNAs and mRNAs in a second modeling approach. We used a stable marriage algorithm to determine the miRNA targets. This approach considered the abundance of each miRNA species in a given cell context to make the prediction.

Results: We identified over 700 miRNA/TF regulatory loops conserved between human and mouse. In particular, we identified two regulatory loops between LMO2 and the miRNAs miR-223 and miR-363 and showed experimentally the existence of these loops. Using hematopoietic stem cells and progenitor cells transplantation experiment we showed that miR-223 and miR-363 are involved in hematopoietic cell fate determination. We also developed a new method for predicting miRNA targets that outperforms the current state of the art tool. This modeling highlighted some unsuspected consequences of miRNA effects such as the cell context specificity of miRNA targets and the cascading effects of RNA abundance modification through the miRNA network. We showed that our modeling can be use to identify miRNAs that are likely linked to cancer through known oncogenes and tumour suppressor genes.

Conclusions/Significance: The results of this research provide a better understanding of the miRNAs' roles through a more accurate identification of their targets. These further highlight the benefits of using computational modelling to the understanding of molecular biology. In particular, with our modelling approaches, we were able to assign a specific function to the miRNAs miR-223 and miR-363 in hematopoiesis. We also highlighted unforeseen consequences of miRNAs regulation, which will improve our ability to use miRNAs as therapeutic agent, in particular in the context of cancer.

1.M. Selbach et al., Widespread changes in protein synthesis induced by microRNAs. Nature 455, 58 (Sep 4, 2008).

2.R. C. Friedman, K. K. Farh, C. B. Burge, D. P. Bartel, Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 19, 92 (Jan, 2009)

3.J. Lu et al., MicroRNA expression profiles classify human cancers. Nature 435, 834 (June 9, 2005).

4.A. Grimson et al., MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol. Cell 27, 91 (Jul 6, 2007).

PO12

Title: Regulation and targeting of telomere maintenance

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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 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 antiproliferative effects with some specificity in cancer cells. G-quadruplex structures have also been identified in the promoter regions of important proto-oncogenes.

Purpose of the study: Our goal is to determine whether G-quadruplex ligands (PIP and CLIP) 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 were subjected to qRT-PCR to evaluate the effect of ligand treatment on the expression level of proto-oncogene c-myc and on hTERT.

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. Preliminary promoter studies showed a decrease in c-myc levels in A549 cells when treated with 0.5X IC50 PIP, with a significant drop occurring at Day 22 and levels remaining lower than in MRC5s. hTERT expression levels were lower in A549 cells treated with 0.5X IC50 PIP.

Conclusion: Our results suggest that G-quadruplex stabilizing ligand PIP can significantly affect telomerase-positive cell proliferation at a dilution of 0.5X IC50. Furthermore, ligand treatment may possibly decrease expression of proto-oncogene c-myc and of hTERT, contributing to the decrease in cell proliferation.

PO13

Title: EEF1A1 is part of the AID cytoplasmic retention complex, which is in dynamic equilibrium with Hsp90.

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Keywords: Antibody diversification, AID, subcellular localization, mutagenesis

Background information: Activation-induced deaminase (AID) is necessary for generating antibody diversification, but must be tightly regulated due to its DNA mutator activity. Deregulated AID expression and activity have been implicated in the generation of oncogenic mutations and chromosomal translocations that can result in the development of B cell lymphomas or leukemia's. We are interested in studying mechanisms regulating AID subcellular localization. Although AID acts in the nucleus, it remains predominantly cytoplasmic due to CRM1 mediated active nuclear export (NE), and cytoplasmic retention (CR), which outcompete its active nuclear import. The mechanism mediating AID CR is unknown, and it is unclear how both NE and CR are implicated in restricting AID nuclear accumulation.

Purpose of the study: Our goal is to understand the importance of CR in regulating AID subcellular localization, to elucidate its mechanism, and to determine the biological importance of CR in limiting AID activity.

Methods: To determine the relative importance of AID CR, we inhibited NE, using the CRM1 inhibitor leptomycin B (LMB), and followed localization of GFP tagged AID, or endogenous AID by immunofluorescence. To define the mechanism of retention, we have investigated the interaction between AID homologs and CR mutants with different cytoplasmic partners of AID by co-immunoprecipitation. Finally, we tested the mutagenic activity of various AID mutants deficient in CR. We tested catalytic activity in transformed E. coli, and somatic hypermutation activity by reconstituting AID-/- DT40 B cells and monitoring mutagenesis.

Results: In human B cell lines, we detected very little AID in the nucleus after LMB treatment, suggesting that CR is more important than NE for restricting AID nuclear accumulation. We also determined that CR is a conserved feature of AID, though different homologs show differential CR strength in mammalian cells. These differences in strength seem to be due to minor structural differences in the C-terminus. Interestingly, these have a profound effect on the interaction of AID and a recently described cytoplasmic partner, eEF1A1. Weaker retention correlates with decreased eEF1A1 binding and vice versa, suggesting that eEF1A1 may mediate CR of AID. Finally, we determined that mutations affecting AID CR result in increased biological activity, demonstrating an important role for retention in limiting AID's mutagenic potential.

Conclusion: Our results indicate that CR is the major force restricting nuclear accumulation of AID, and that it is an evolutionarily conserved feature of AID with variable strength amongst homologs. These results would suggest that CR has an important role in regulating the biological activity of AID, and we were able to confirm this, since AID mutants deficient in CR show significantly increased biological activity. Furthermore, we show direct correlation between CR strength, and eEF1A1 binding, strongly suggesting that eEF1A1, a highly expressed protein translation factor, is involved in mediating AID CR. Future studies will look to further characterize the interaction of AID and eEF1A1 to understand the dynamics of this interaction.

PO14

Title: Potential role of CXCR7 as a negative modulator of leukemia 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. 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 SDF-1 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 CXCR4-mediated migration and re-sensitization capacity.

Methods: All our experiments have been performed on leukemia cell lines (REH, 697 or K562). The overexpression has been achived by the Amaxa NucleofectorTM technology. Dose-response migration experiments have been done on ChemotoX® Disposable Chemotaxis System. The migration potency has been evaluated using a TC10TM automated cell counter following 3h incubation at 37oC, 5% CO2. The expression of CXCR4 and CXCR7 has been evaluated by flow cytometry using fluorochrome-coupled monoclonal antibody. During the recycling kinetics, cycloheximide has been added to block the cellular traduction.

Results: Our results suggest that the chemotaxis potency mediated by SDF-1 is governed by the ratio of CXCR4/CXCR7 expression rather than by the expression of CXCR4 alone. We also show that the presence of CXCR7 impinges on the recycling capability/re-sensitization of CXCR4. This last results suggest that the fate of CXCR4 following a SDF-1 stimulation would be in part controlled by the expression of CXCR7.

Conclusions: CXCR4-mediated chemotaxis/migration response results from a persistent receptor activation/intracellular signaling. Taken together, our results would 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. We will assess this question using human xenograft in mice**.** This model will be presented.

PO15

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 TRAF1-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.

PO16

Title: Aryl-hydrocarbon receptor activation induces differentiation of primary myeloid leukemia stem/progenitor cells.

Author: Caroline Pabst

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Keywords: Actue myeloid leukemia, leukemic stem cell, Aryl-hydrocarbon receptor, chemical screening

Background information: Understanding the biology of acute myeloid leukemia (AML) stem/progenitor cells is a crucial prerequisite for the development of more efficacious therapeutic strategies as current standard therapies fail to permanently eradicate these cells in a significant proportion of patients. AML is a heterogeneous disease consisting of cells with distinct characteristics caricaturing the hierarchy of the normal hematopoietic system. Leukemia initiating cells (LIC) which are defined by their potential to engraft immunocompromised mice are at the apex of this hierarchy and share some critical features with normal hematopoietic stem cells as the ability to self-renew. However, LICs differentiate or undergo apoptosis when deprived of their in vivo environment, which is a major obstacle in the development of LIC targeted therapies and casts doubt on the interpretation of results emanating from ex vivo treatment of these cells.

Purpose of the study: Defining mechanisms which induce differentiation and apoptosis of AML stem/progenitor cells and identifying tools to prevent these events would facilitate the in vitro manipulation of cells of interest, permit screening for anti-leukemic drugs on self-renewing AML cells instead of differentiating blasts and unravel key networks controlling LIC self-renewal, which might itself lead to identification of therapeutic targets.

Methods: We have taken a flow-cytometry based chemical screening approach to identify compounds with the ability to prevent differentiation of primary myeloid leukemia cells in vitro. Selected compounds were first tested on a subset of AML specimens with different morphology and genetic aberrations and further validated in functional in vitro and in vivo assays. For in vivo experiments primary human AML cells were transplanted into immunocompromised NSG-mice either freshly or after ex vivo culture in presence of selected compounds or control conditions. Human leukemic engraftment was detected by flow cytometry in mouse bone marrow 10-16 weeks after transplantation.

Results: We identified suppressors of the Aryl-hydrocarbon receptor (AhR) pathway as predominant group of compounds emerging from the screen. We found that the AhR itself is well expressed in fresh AML cells but not its target genes. The pathway was strongly activated within 24 hours following in vitro culture. Inhibition of this activation by AhR-antagonists partially to completely rescued loss of CD34+ expression in all tested samples and allowed expansion of CD34+CD15- cells in cases where we observed a net loss in control conditions. Loss of leukemia initiating cells was partially but in no case completely rescued by the AhR-antagonist SR1 in six tested AML samples. The compound UM1 which does not suppress AhR-target genes showed effect on its own in ~50% of samples in in vitro and in vivo assays and further enhanced the effect of AhR-suppressors in some cases when administered simultaneously.

Conclusion: The AhR-pathway is rapidly and strongly activated upon in vitro culture of primary human AML samples and this activation induces differentiation of AML stem/progenitor cells, which can be partially rescued by AhR-antagonists. More pathways, one of which is potentially targeted by the compound UM1, need to be identified to achieve a full maintenance/expansion of primary AML stem/progenitor cells in vitro.

PO17

Title: Modifications of histone methylation can modulate hematopoietic stem cell fate decisions.

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Keywords: epigenetic; histone demethylases; hematopoietic stem cells; self-renewal; myeloid leukemia

Background information: The importance of epigenetic regulation of hematopoietic stem cell (HSC) fate is illustrated by the MLL gene. This gene, encoding a histone methyl transferase of lysine 4 on histone 3 (H3K4), is essential for HSC and is also rearranged in 70% of infant leukemia. A class of enzymes, the histone demethylases (HDMs), also appears critical for HSCs and is increasingly involved in hematologic malignancies. An in vivo functional screen of HDMs in mice using small interfering RNAs (siRNAs), allowed us to identify Jarid1b, an H3K4 demethylase, as a potential repressor of genes essential for maintaining stemness: its repression leads to HSC expansion in vitro.

Purpose of the study: The aim of my research project is to confirm the importance of HDMs in HSC fate modulation in normal human hematopoiesis and in pediatric acute myeloid leukemia (AML).

Methods: We performed a comprehensive expression profile of HDMs using qRT-PCR in cells purified from cord blood units (Lin-CD34+ HSCs and differentiated cells) and in AML cells from the cell line MONO-MAC-1 (with translocation t(9;11)). In parallel, a transcriptome sequencing (RNA-seq) study was performed using cord blood Lin-CD34+ HSCs and differentiated cells, and primary leukemic cells obtained from pediatric AML samples with the t(9;11). RNAi mediated knockdown of Jarid1B in mouse HSCs was also performed to study changes in expression profiling relative to control cells.

Results: The results from both expression studies showed a similar differential expression of HDMs between HSCs and differentiated cells in human cord blood, including for JARID1B. Comparable expression differences were also observed for some of these enzymes between leukemic and normal cells. Knockdown of Jarid1b in mouse HSCs led to up-regulation of stem/progenitor associated genes, such as Hoxa7, Hoxa9, Hoxa10, Hes1, Gata1, Gata2 and Msi2.

Conclusion: These expression studies confirm that some candidate HDM genes are differentially expressed in human HSCs compared to mature and leukemic cells. Knockdown of Jarid1b in mouse HSCs leads to upregulation of “stemness” associated genes and increased self-renewal in culture. As Jarid1b is preferentially expressed in the stem/progenitor cell compartment in both mouse and human HSCs, its inhibition will now be tested as a potential cord blood HSC expansion strategy in vitro. This could lead to improved safety and availability of HSC transplantation procedures in oncology. In addition, HDM transcripts that are most abundant in leukemic cells will be similarly targeted to evaluate their role in leukemia maintenance, with potential therapeutic applications.

PO18

Title: Role of Miz-1 (Myc-Interacting Zinc-finger protein-1) as a regulator of p53 dependent pathways during pre-TCR selection at the DN3 β selection checkpoint

Author: Marissa Rashkovan

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Keywords: Miz-1, p53, T cell development, β-selection

Background Information: The Myc-Interacting Zinc-finger protein-1 (Miz-1) can bind to the proto-oncogene c-Myc and enables a Miz-1/c-Myc complex to regulate the transcription of specific target genes. Miz-1 contains a BTB/POZ domain and thirteen zinc finger domains that enable it to bind to DNA and to interact with a number of partner proteins in addition to c-Myc. While Miz-1 itself functions as a transcriptional transactivator, it enables c-Myc to act as a transcriptional repressor of genes negatively regulating cell cycle progression. We have previously reported that Miz-1 plays an essential role during early stages of T cell development. We have found that Miz-1 regulates the IL7R pathway by regulating the Jak/STAT inhibitor SOCS1 in DN1 and DN2 pre-T cells. In addition to this, we have accumulated evidence that Miz-1 exerts a role in DN3 pre-T cells at the so called β-selection checkpoint, i.e. in cells in which V(D)J recombination of T cell receptor genes is ongoing to produce the beta chain of the pre-T cell receptor (pre-TCR) and where the DN3 cells that have undergone a productive rearrangement and express the pre-TCR are selected. Our data suggest that during V(D)J recombination, Miz-1 is required to restrain p53 activity, notably its ability to induce apoptosis through the up-regulation of such genes as Bax, Noxa or Puma. This is supported by our finding that in the absence of a functional Miz-1 i.e. in Miz-1ΔPOZ mice, pre-TCR bearing cells are lost from the thymus by apoptosis, have high levels of Bax, Noxa and Puma and a slower progression through the cell cycle, causing a very low thymic cellularity.

Purpose of the study: Our goal was to further characterize the role of Miz-1 in the p53 pathway at the β-selection checkpoint and determine how loss of Miz-1 affects pre-TCR selection.

Methods: In this study, we made use of mice lacking a functional Miz-1 (Miz-1ΔPOZ), which have severe defects in T cell development. Crossing these mice with mice deficient in p53 allowed us to assess the role of Miz-1 as a regulator of p53 dependent pathways, specifically at the β-selection checkpoint. Furthermore, we used ChIP-seq and microarray experiments to determine possible targets of Miz-1 in DN3 pre-T cells, including Rpl22, which can act as a regulator of p53 activity in pre T-cells. We then used ChIP-PCR and luciferase experiments to confirm the regulation of the Rpl22 promoter by Miz-1 in vitro.

Results: Our experiments indicated that Miz-1 deficiency results in increased expression of p53 target genes, including Puma, Noxa, Bax and p21. Deletion of p53 in these mice induces down-regulation of most of these target genes. Strikingly, deletion of p53 rescues the increased apoptosis and developmental block seen in the Miz-1-deficient DN3 pre-T cells at the β-selection checkpoint. Miz-1ΔPOZ x p53-/- mice have now almost normal thymic cellularity and show normal pre-T cell development. Using ChIP-seq data, we were able to identify Miz-1 binding sites in the promoter regions of several genes, including Rpl22. Rpl22 has previously been shown to play a role in pre-T cell development, specifically at the β-selection checkpoint, by regulating the levels of p53 target gene expression. This activity of Rpl22 is very likely mediated by regulating the expression or stability of the p53 protein itself. We have been able to confirm that this gene is indeed misregulated in Miz-1-deficient DN3 pre-T cells, and we can show that Miz-1 occupies the promoter of Rpl22 in these cells. Furthermore, we can show that Miz-1 activates the Rpl22 promoter using reporter gene assays.

Conclusion: Our results suggest that activation of p53 has to be controlled when V(D)J recombination occurs and that Miz-1 can exert such a control on p53. We hypothesize therefore that Miz-1 is necessary to protect pre-T cells from premature apoptosis by restricting a p53-mediated DNA damage response that is possibly initiated by double strand breaks that occur during V(D)J recombination.

PO19

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. Finally, we performed CHromatin ImmunoPrecipitation (CHIP) using different mutants of SMC4 to study the dynamism of condensin on chromatin during the cell cycle.

Results: We discovered by mass spectrometry that Smc4 is phosphorylated on 6 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. Absence of phosphorylation or constitutive phosphorylation alters the binding of condensin on chromatin.

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.

PO20 *(absent / not present)*

Title: Elucidating the structure of the catalytic ATP binding domain from human mTOR

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Keywords: Cancer, mTOR, mRNA Translation, Drug Design

Background: Multiple clinical studies in patients suffering from leukemia and lymphoma established the therapeutic value of mTOR inhibition using the drug Rapamycin, a naturally occurring allosteric inhibitor of mTORC1, and its analogues (Rapalogues). Although promising, a limitation of these inhibitors is their inability to completely suppress mTORC1 signaling. Alternatively, compounds targeting another domain within mTOR, the catalytic ATP-binding site, were designed to overcome these issues. In the pre-clinical setting, these active site mTOR inhibitors (asTORIs) exhibit strong anti-leukemogenic effects and are being transitioned to clinical trials. Currently, the structure of ATP-binding site of mTOR is still unknown and its elucidation in combination with rational drug design is essential to improve the selectivity, potency, and pharmacological properties for this class of inhibitors.

Purpose of Study: Determining the three-dimensional structure of the catalytic ATP binding domain of mTOR will provide a framework for rational structure based design.

Methods and Results: Human mTOR is a ~2500 residue protein and the ATP-binding domain was mapped to a 280 residue region located near its C-terminus. This domain was cloned in various vectors and expressed in different hosts (E. coli, insect cells) to ensure maximum expression and solubility. Purification techniques, such as affinity chromatography and FPLC, were used to isolate the pure protein. Our future work will focus on obtaining the 3-dimentional structure by either NMR spectroscopy or X-ray crystallography. Complex structures will also be solved with existing asTORis. The kinetic and thermodynamic binding properties between each compound and the catalytic domain will be analyzed using isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR).

Conclusions: Overall, we established a purification method to isolate the mTOR catalytic ATP-binding site. Due to high sequence similarity, this structure will likely be similar to kinase domain found in the family of PI3K kinases. Using established strategies on designing de novo inhibitors from structures of other PI3K domains, our results will effectively enable us to rationalize and predict compounds that may improve selectivity and potency for the mTOR catalytic domain.

PO21

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

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Keywords: T-ALL, EZH2, PRC2, Leukemia, JARID2.

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 knock-out mouse model. The Cre-mediated deletion generates a mutated $Ezh2^{\Delta}$ allele and abrogates production of EZH2 protein specifically in 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 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 γδ T cell leukemia (T-ALL) occurrence in mice with bi-allelic deletion of *Ezh2.* Interestingly, lymphoblasts were exclusively positive for the cell surface TCRγδ. Cell cycle analysis on pre-leukemic mice revealed an activation of the G2/M checkpoint. Moreover we found that the Ezh2 deficient TCRγδ 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.

PO22 *(absent / not present)*

Title: Ras-MAPK signaling and chromatin remodeling are key driver pathways in childhood pre-B acute lymphoblastic leukemia

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Affiliation: Dr Daniel Sinnett, Sainte-Justine UHC Research Center, University of Montreal, Montreal, Qc, Canada

Background information: Despite significant advances in our understanding of the pathobiology of acute lymphoblastic leukemia (ALL), the most frequent pediatric cancer, its etiological causes remain elusive. While gross somatic chromosomal alterations play an important role in driving leukemogenesis, they are insufficient on their own to cause cellular transformation, suggesting the involvement of additional cooperating events for childhood leukemogenesis. Direct evidence that childhood ALL has a genetic component is provided by the high risk of developing the disease associated with certain inherited disorders such as Bloom's syndrome, Down syndrome, Fanconi anemia, neurofibromatosis and ataxia telangiectasia. The influence of heredity on susceptibility to ALL outside these syndromes, which represent less than 5% of all pediatric ALL cases, is largely undefined. The frequency and impact of rare variants and somatic-specific mutations, and how germline and somatic-specific events jointly act to disrupt biological pathways and contribute to the onset and the progression of ALL are unknown.

Purpose of the study: Our goal was to identify driver genes/pathways of childhood ALL and to provide the first clues of interplay between the constitutional and somatic genomes that could contribute to leukemic transformation.

Methods: Using next generation technology, we deep-sequenced the whole exomes of 29 childhood ALL quartets consisting of patient-matched normal (in remission) and tumor samples (at diagnosis) and both parents. Patients were representative of the three major molecular subtypes (hyperdiploidy, chromosomal rearrangements, cytogenetically normal) of pre-B ALL. Incorporating parental sequence information allowed us to reduce sequencing errors and facilitate the identification of inherited variants and somatic-specific mutations. An integrative bioinformatics strategy and a data reduction pipeline were developed to identify variants (germline and somatic) that were more likely to have a functional impact and classified them according to their leukemogenesis driving potential.

Results: We identified somatically acquired mutations in a limited number of driver pathways common to all molecular subtypes. Key regulators of Ras-MAPK signalling (FLT3, BRAF, NF1, NRAS) and chromatin remodeling pathway (CREBBP, WHSC1, DOT1L) were among the most frequent targets. We also identified rare inherited damaging variants within these pathways suggesting that both causal somatic and germline events leading to the aberrant Ras activation or the misregulation of epigenetic controls might constitute ALL drivers.

Conclusion: Leveraging the power of this unique quartet design, we generated a comprehensive repertoire of private and recurrent somatic gene mutations as well as rare inherited germline variants that are candidate drivers for childhood ALL. Targeted genes clustered in key cancer-related pathways, including Ras-MAPK signaling and chromatin remodeling. Our results suggest an interplay between the constitutional and the somatic genomes that contributes to drive leukemic transformation. Ultimately, this work will provide invaluable insights to understanding the mechanisms underlying pediatric ALL and eventually identifying potential new targets for antileukemic agents.

PO23

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 morbidity and mortality for children and adults with acute myeloid leukemia (AML). These patients are at particularly high risk of infection, likely because they receive high doses of chemotherapeutic agents that result in prolonged and severe neutropenia. Current anti-fungal therapy is challenged by the emergence of multidrug resistant fungi or high toxicity adverse effects; therefore, there is an urgent need for new antifungal strategies. Histone H3 lysine 56 acetylation (H3K56ac) and deacetylation play important roles in chromosome assembly during DNA replication and DNA repair in fungi. We recently demonstrated that genetic inactivation or pharmacological inhibition of the fungal-specific histone deacetylase (HDAC) Hst3 results in H3K56 hyperacetylation and lethality in the most frequently encountered human fungal pathogen Candida albicans. This indicates that Hst3-mediated H3K56 deacetylation represents a promising pathway for novel anti-fungal drug discovery.

Purpose of the study: Our goal is to discover small molecules that affect 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 chemical library containing 20,000 structurally diverse molecules (ChemBridge DIVERTSet). Small molecules that selectively inhibit proliferation of wild type (WT) C. albicans but not mutant cells lacking H3K56 acetylation are considered as primary hits. The rationale is that inhibitors of Hst3 (HDAC inhibitors) are only cytotoxic to cells that can acetylate H3K56. These hits will be validated individually and the effect of these compounds on the levels of H3K56ac will be examined.

Results: From our chemical screen, we identified 11 compounds that inhibit at least 80% of the growth of WT cells but allow 100% growth of the mutant cells lacking H3K56ac. We individually tested these compounds by performing concentration inhibition curves and confirmed that 5 of them exhibited the expected growth inhibition profile. Interestingly, these 5 compounds have similar chemical scaffold with inhibitory concentrations that range between 3 and 25 micromolar. We are currently evaluating the effect of these compounds on H3K56 acetylation by immunoblotting. Finally, the change in H3K56ac levels will be assessed by quantitative mass spectrometry.

Conclusion: From a chemical library of 20,000 compounds, we identified and validated 5 compounds that likely target the Hst3-mediated deacetylation pathway. We are currently trying to identify the cellular targets of these compounds.

PO 24

Title: Suppression of Eukaryotic Initiation Factor 4A Chemosensitizes Myc driven lymphomas to ABT-737 therapy

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Keywords: eIF4A, translation initiation, ABT-737, Chemosensitization

Background information: One of the most altered pathways in human cancers is the PI3K/Akt/mTOR signaling axis, which exerts profound regulation over protein synthesis. This is achieved by controlling the assembly of eukaryotic initiation factor (eIF) 4F, a complex consisting of the cap binding protein, eIF4E; an RNA helicase, eIF4A; and a scaffolding protein, eIF4G. EIF4A exists as two different isoforms and we have recently demonstrated that these perform different roles in translation. Suppression of eIF4AI, but not eIF4AII, is able to curtail cell proliferation in Myc-driven lymphomas. An increasing concern in cancer therapy is chemoresistance of tumor cells which can be attributed to evasion of apoptosis through increased levels of pro-survival proteins (eg. Bcl-2, Mcl-1). The compound ABT-737 can cause tumor regression, targeting Bcl-2 and two other Bcl-2 family members, Bcl-XL and Bcl-W. However, resistance to ABT-737 can arise because of elevated levels of other Bcl-2 family proteins, like Mcl-1 which bind poorly to ABT-737.

Purpose of the study: Mcl-1 is a translationally regulated mRNA transcript. We aimed to evaluate whether suppression of Mcl-1 inhibition through eIF4A suppression in an in vitro lymphoma model could resensitize lymphoma cells to ABT-737.

Methods: The increased level of Mcl-1 is a major cause of ABT-737 resistance. Mcl-1 has a short protein half life (<1 h) and it is regulated by the P13K/mTOR signaling pathway - inhibition of protein synthesis results in a rapid decline in Mcl-1 protein. Taking this into consideration, we proposed a strategy to inhibit both Mcl-1 and Bcl-2 proteins. We took advantage of RNAi to specifically suppress each eIF4A isoform and combined this with the inhibitor ABT-737 in Arf-/-Eμ-myc/Bcl-2 and p53-/-Eμ-myc/Bcl-2 lymphoma cells (the Eμ-myc model is based on the same genetic lesions found in human Burkitt's lymphoma). We determined the consequences of these treatments on cell proliferation and cell death

Results: We found that short-term suppression of eIF4AI, but not eIF4AII, affects cell proliferation in Arf-/-Eμ-myc/Bcl-2 cells. We also observed that long-term suppression of eIF4AI was lethal to cells. On the contrary, we observed that these results were blunted in p53/-Eμ-myc/Bcl-2 cells. Suppression of eIF4AI and Mcl-1 (control) in the presence of ABT-737 led to a dose dependent reduction in cell viability. This appeared to be a p53-dependent phenomenon since no significant inhibition was observed when eIF4AI/II was suppressed in p53-/-Eμ-myc/Bcl-2 cells.

Conclusion: Our experiments demonstrate that suppression of eIF4AI isoform confers a growth disadvantage to Arf-/-Eμ-myc/Bcl-2 lymphoma cells and is able to synergize with ABT-737. This effect was p53 dependent.

PO25

Title: Integrative approach to discover molecular events transforming pre-leukemic thymocytes into leukemic T-ALL cells

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Keywords: SCL, LMO, NOTCH1, T-ALL.

Background information: The study of the molecular mechanisms underlying T-ALL development in both mice and human strongly supports the view that transformation of normal thymocytes into leukemic cells is a stepwise process involving primary (initiating) and secondary (collaborating) events. Chromosomal rearrangements mark initiating events that reprogram normal thymocytes into a pre-leukemic state, whereas at a later stage pre-leukemic thymocytes acquire collaborating events leading to full malignant transformation. Using the SCL-LMO1 transgenic mouse model, our lab has previously shown that efficient induction of T-ALL by SCL and LMO1 oncogenes (primary event) requires constitutive Notch1 signalling and a functional pre-TCR (secondary events). Gain of function mutations of the NOTCH1 gene are found in more than 50% of T-ALL. However, collaborating events not involving NOTCH1 remain to be discovered.

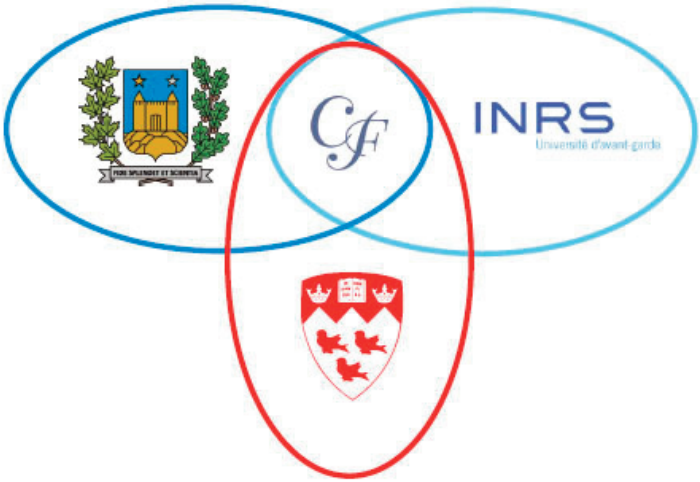
Purpose of the study: Herein, our goal is to discover novel molecular events that are capable of transforming pre-leukemic SCLtgLMOtg thymocytes into leukemic cells in the absence of Notch1 gain of function mutations.

Methods: We use the Cd3-/-SCLtgLMO1tg mouse model of T-ALL to find secondary molecular events that collaborate with SCL and LMO1 in leukemia progression. Importantly, pre-leukemic Cd3-/-SCLtgLMO1tg thymocytes are able to progress to a leukemic state but do not acquire gain of function mutations in the Notch1 gene due to deficient pre-TCR signalling. Therefore, the Cd3-/-SCLtgLMO1tg mouse model represents a suitable model system to uncover mechanisms of T-ALL development that are not dependent on Notch1 gain of function mutations. Our approach to identify collaborating events involves the characterization of Cd3-/-SCLtgLMO1tg leukemias using complementary high-throughput techniques, including RNA-seq, microarrays and phosphoproteome analysis.

Results: Phosphoproteome analysis identified top activated kinases and corresponding signalling pathways that might be implicated in the leukemia progression, including CkII alpha (proliferation), Cdk2 (cell cycle), p70S6K (mTOR pathway), and Pim2 (evasion of apoptosis). Additionally, the transcriptome analysis identified several strategies by which Cd3-/-SCLtgLMO1tg thymocytes progress to a disease state, including (i) inhibition of Smad signalling by down-regulation of Smad4/5, (ii) up-regulation of cell cycle genes (Cdc25b, Ccne2, Cdk2), (iii) up-regulation of T-cell activation genes associated with the pre-TCR and thymocyte proliferation (Zap70, Thy1, Nck2, Sit1, Cd3d, and Cd2). Overall, the combined transcriptome and phosphoproteome analyses indicate that the transition from pre-leukemic to leukemic stage in the absence of Notch may be a result of up-regulation of mTOR signalling and Smads down-regulation.

Conclusions: We have identified gene signatures and activated kinases that are implicated in the transformation of Cd3-/-SCLtgLMO1tg thymocytes into leukemic cells in the absence of Notch1 mutations. Furthermore, the ongoing characterization of genetic alterations (mutations) in Cd3-/-SCLtgLMO1tg leukemias by deep-sequencing will complement this effort and will allow us to obtain a comprehensive catalog of secondary events that collaborate with the major oncogenes for leukemia onset. Finally, the T-ALL induction potential of collaborating events will be tested by retroviral-mediated gene transfer in T progenitors.

Notes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