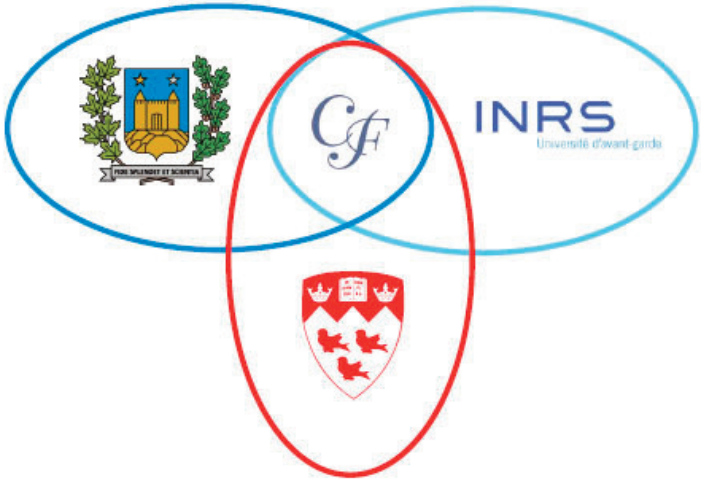
Cole Foundation
"Research Celebration Day"
May 11, 2012



Journée « Célébrons la recherche »
de la Fondation Cole
11 mai 2012

The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD programmes to promote research in pre-leukemia, leukemia, and other leukemia-related diseases in children and young adults, as well as the development of clinical care for patients affected by these diseases. With the announcement of 2012 Fellows, the Fellowship programme has supported more than 90 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 $5 million has been committed to this programme.

The Cole Foundation was established in 1980 by John N. (Jack) Cole as a private family foundation to provide funds for medical facilities concerned with child care and research towards a cure for leukemia. The Foundation also awards grants to worthy community causes. The catalyst for the Foundation's creation was the death of his daughter, Penny Cole, from leukemia over a decade earlier.

Through the Foundation, Jack Cole provided millions of dollars for medical facilities in Montreal. Notable among his achievements was the support of the Penny Cole Laboratory at the Montreal Children's Hospital and the establishment of the Jack Cole Chair in Pediatric Oncology and Hematology at McGill University.

Since his death in 2004, the Foundation has continued to carry on Jack Cole's mission with a renewed focus on research and child care for young patients afflicted with leukemia and leukemia-related diseases.

La Fondation Cole

La Fondation Cole soutient la recherche sur le syndrome myélodysplasique, la leucémie et d'autres affections liées à la leucémie chez les enfants et les jeunes adultes, ainsi que le développement des soins cliniques pour les personnes atteintes de ces maladies, en offrant des bourses à des chercheurs-cliniciens, des résidents postdoctoraux et des étudiants diplômés inscrits à des programmes de doctorat. Avec l'annonce des boursiers 2012, le programme a appuyé plus de 90 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 de cinq millions de dollars y ont été consacrés.

La Fondation Cole a été instituée en 1980 par John N. (Jack) Cole à titre de fondation familiale privée pour subventionner des services de santé s'intéressant aux soins et à la recherche en pédiatrie et à la découverte d'un traitement curatif pour la leucémie. Elle a été créée à la mémoire de sa fille, Penny Cole, emportée par la leucémie plus de dix ans avant. La Fondation appuie aussi des causes communautaires louables.

Grâce à la Fondation, Jack Cole a donné des millions de dollars à des services de santé de Montréal. Parmi ses grandes réalisations figurent son soutien du Laboratoire Penny Cole à l'Hôpital de Montréal pour enfants et la création de la chaire Jack Cole en oncologie-hématologie pédiatrique à l'Université McGill.

Depuis le décès de son maître d'œuvre en 2004, la Fondation poursuit sa mission avec un intérêt marqué pour la recherche et les soins pédiatriques destinés à de jeunes patients affectés par la leucémie ou une maladie liée à la leucémie.

Program/Programme

9:30 - 10:45 AM	Poster Set-up Installation des affiches
10:00 - 10:30 AM	Round Table (new fellows) Table ronde (nouveaux boursiers) *Holmes Hall, 3605 de la Montagne Street* *Holmes Hall, 3605, rue de la Montagne*
10:45 AM - 12:00 PM	Poster Session Part 1 Séance d'affichage, 1re partie
11:30 - 1:30 PM	Lunch / Dîner
12:30 - 1:45 PM	Poster Session Part 2 Séance d'affichage, 2e partie
2:00 - 3:15 PM	Lecture / Conférence *Martin Amphitheater, McIntyre Building* *Amphithéâtre Martin, Pavillon McIntyre*
Welcome / Bienvenue	*Barry Cole* *President / Président* *Cole Foundation*
Introduction / Introduction	*Dr. Janusz Rak* *Professor of Pediatrics & Jack Cole Professor in Pediatric Oncology, McGill University* *Professeur de pédiatrie et titulaire de la chaire Jack Cole en oncologie pédiatrique, Université McGill*
Keynote Speaker / Conférencier invité	*Dr. Connie Eaves, Ph.D., FRSC,* *Distinguished Scientist, Terry Fox Laboratory, BC Cancer Agency* *Professor of Medical Genetics, University of British Columbia*
3:15 - 3:30 PM	New fellows and announcement of prizes Nouveaux boursiers et remise des prix
3:30 PM	Reception / Réception

The 2012 - 2014 Cole Foundation Fellows
Les boursiers de recherche de la Fondation Cole 2012 - 2014

McGill University

Daphne Dupéré-Richer, PhD program
Supervisor: Wilson Miller, Jr., Lady Davis Institute, Experimental Medicine
Project title: Molecular mechanism of acquired resistance to histone deacetylase inhibitors in lymphoma cells
Description: Histone deacetylases inhibitors (HDACi) are emerging has a promising therapy against acute leukemia which is the most common type of pediatric cancer. However, as in any therapy, resistance is inevitable. My project focuses on the role of autophagy in resistance to HDACi therapy. My data suggests that inhibition of autophagy may overcome acquired resistance to HDACi.

Yi Fang, Post PhD program
Supervisor: Janusz Rak, Montreal Children's Hospital, Pediatric Oncology & Hematology
Project Title: Microvesicles as mediators and messagers of leukemogenesis in pediatric acute myeloid leukemia (pAML)
Description: Acute myeloid leukemia (AML) has poorer outcomes in children. We will analyze how AML oncogenic molecules are captured by microvesicles (oncosomes) and transferred to normal cells, and whether this process contributes to leukemogenesis and drug resistance. Thus, new drugs and diagnostics can be developed to exploit oncosomes in childhood AML.

Johanna Mancini, PhD program
Supervisor: Chantal Autexier, Lady Davis Institute, Experimental Medicine
Project Title: Targeting Telomeres and Telomerase in Cancer and Leukemia using G-quadruplex Ligands
Description: Pediatric Leukemia affects children during a critical period of growth and development. Current chemotherapy treatments target all rapidly dividing cells, causing terrible side-effects. Stabilization of G-quadruplex structures within telomeres and promoter regions of cancer genes provides an opportunity for targeted therapy via telomerase inhibition and/or suppression of oncogene expression, thereby reducing unnecessary side-effects.

Université de Montréal

Gloria Assaker, PhD program
Supervisor: Gregory Emery, IRIC, Molecular and Cell Biology
Project Title: A genome-wide screen for regulators of ligand activity in Notch signaling
Description: Pediatric T-cell acute lymphoblastic leukemia (T-ALL) is primarily caused by aberrant activation of the Notch pathway. Notch signalling requires interaction between Notch receptor and its ligand Delta. Delta regulation is still poorly understood. This projects aims at identifying new regulators of Delta, and thus potential therapeutic targets for pediatric T-ALL.

Nicolas Montpas, PhD program
Supervisor: Nikolaus Heveker, CHU Ste-Justine, Molecular and Cell Biology
Project Title: The role of CXCR7 as a negative modulator of leulemia bone marrow niche homing
Description: Childhood leukemia cells can migrate towards specific regions of the bone marrow, called “niches”, that provide resistance to chemotherapy. Our study will test if bone marrow homing can be predicted, using the recently discovered chemokine receptor CXCR7 as a marker. This may ultimately permit chemotherapy adjustment for pediatric leukemia patients.

Eustache Oussa, PhD program
Supervisor: Laurent Sabbagh, Hôpital Maisonneve-Rosemont, Immunology
Project Title: Rôle de TRAF1 dans la survie des leucémie et lymphomes
Description: Mon projet de doctorat vise à caractériser la coopération entre les protéines TRAF1 et TBK1 menant à la survie de T-ALL , la leucémie la plus commune chez les enfants de moins de 15 ans. Il permettra l'identification de nouvelles molécules et des inhibiteurs capables d’affecter les activités des kinases impliquées dans la survie des lymphocytes à des fins thérapeutiques.

Caroline Pabst, Post PhD program
Supervisor: Guy Sauvageau, IRIC, Medicine
Project Title: Identification of self-renewal agonists of primary human AML stem cells
Description: The major aim of the project is to identify pathways regulating self-renewal of acute myeloid leukemia stem cells in children and young adults by using a chemogenomic approach. New insights into these networks are the prerequisite to develop novel therapeutic agents to treat pediatric leukemia more efficiently.

Xavier Robellet, Post PhD program
Supervisor: Damien D'Amours, IRIC, Molecular and Cell Biology
Project Title: Rôle du complexe condensine dans l'intégrité génomique et la multiplication cellulaire
Description: Le virus d'Epstein Barr (VEB) prédispose les enfants au lymphome de Burkitt. Ce virus est connu comme un agent qui dérégule le complexe condensine dans les cellules. Mes travaux consisteront à investiguer comment une activité kinase du VEB affecte la régulation de condensine et comment l'inhibition de condensine pourrait prévenir la lymphogénèse.

Sarah Tsao, Post PhD program
Supervisor: Alain Verreault, IRIC, Molecular and Cell Biology
Project Title: Inhibition of histone deacetylation as a novel antifuntal therapy in leukemia patients
Description: Children with acute leukemia have increased risk for life-threatening fungal infections. Development of new antifungal therapies is necessary due to limitations of current treatments. We showed that the deacetylase for histone H3K56 is a promising drug target. I will use high-throughput chemical screening to identify potent deacetylase inhibitors to treat fungal infections.

Diogo Veiga, Post PhD program
Supervisor: Trang Hoang, IRIC, Hematopoiesis & Leukemia Research Unit
Project Title: Multi-level approach to discover genetic mutations that drive T-cell transformation
Description: T-cell acute lymphoblastic leukemia (T-ALL) is a common pediatric cancer. Current treatment protocols are efficient but non-discriminatory and cause serious side effects that have life-long consequences, underscoring the need to develop novel targeted therapies. We will apply an innovative approach combining computational and experimental methods to find mutations associated with leukemia initiation and self-renewal properties of leukemic stem cells. The findings of this project may have a great impact on the development of new therapeutic strategies in T-ALL.

École Polytechnique de Montréal

Mohamed Aissiou, PhD program
Supervisor: Delphine Périé-Curnier, École Polytechnique de Montréal, Biomedical Engineering
Project Title: Leucémie aiguë chez l'enfant : détection précoce de la cardiotoxicité de la doxorubicine par IRM multi-paramétrique
Description: Doxorubicin chemotherapy is often used to treat children with acute lymphoblastic leukemia. However, its effectiveness is hampered by a cohort of adverse effects with the most notable one being its degenerative cardiotoxicity. A new MRI protocol is proposed with the aim of characterizing the biochemical and structural changes of the heart tissue.

La doxorubicine est souvent utilisée dans le traitement chimiothérapeutique des leucémies lymphoblastiques aiguës chez l'enfant. Toutefois, son efficacité se heurte à une cohorte d'effets indésirables dont le plus notable est sa cardiotoxicité dégénérative. Un nouveau protocole IRM est proposé dans le but de caractériser les changements biochimiques et structurels du tissu cardiaque.

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

Presenters / Liste des exposants

Ashton-Beaucage, Dariel
Bisaillon, Richard
Carli, Cédric
Davoudi, Sayeh
Doucette, Kimberley
Fournier, Marilaine
Gauthier, Simon-David
Gerby, Bastien
Hariri, Fadi
Lisi, Veronique
Methot, Stephen
Neveu, Benjamin
Nichol, Jessica
Nielsen, Torsten
Orthwein, Alexandre
Pécheux, Lucie
Rashkovan, Marissa
Siddiqui, Nadeem
Simon, Camille
Spinella, Jean-Francois
Vasquez, Gabriela
Vincent, Krystel
Wang, Xue Qing David

Poster List / Liste des projets exposés

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PO2	Bisaillon, Richard PhD program	C-TERMINAL DOMAIN OF MEIS1 CON VERTS PKNOX1INTO A HOXA9-COLLABO RATING ONCOPROTEIN
PO3	Carli, Cédric Post PhD program	INVOLVEMENT OF TGF-B IN THE CREATION OF AN IMMUNOSUPPRESSIVE MICROENVIRONMENT DURING LEUKEMIA.
PO4	Davoudi, Sayeh PhD program	THE ROLE OF TRANSLESION SYNTHESIS POLYMERASE REV1 IN GENERATION OF POINT MUTATIONS IN MAMMALIAN CELLS
PO5	Doucette, Kimberley Masters program *(absent)*	THE EFFECT OF ORGANIC SOLVENTS AND CYP1A1, CYP2E1, GSTM1 POLYORPHISMS ON THE DEVELOPMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA IN QUEBEC CHILDREN

PO6	Fournier, Marilaine PhD program	COMPARISON OF THE OVEREXPRESSION OF HOXA4 AND HOXB4 IN HEMATOPOIETIC STEM AND PROGENITOR CELLS IN VITRO AND IN VIVO
PO7	Gauthier, Simon-David PhD program	DIMINISHED INTERLEUKINE-7 LEVELS AND LOW DENDRITIC CELL COUNTS CONTRIBUTE TO CONSTRAIN HOME SOTATIC PERIPHERAL EXPANSION OF CD4+ T CELLS DURING GRAFT-VERSUS-HOST DISEASE.
PO8	Gerby, Bastien Post PhD program	EXPLORING THE ROLE OF SCL, LMO1 AND NOTCH1 ONCOGENES IN PRE-LEUKEMIC T CELL DEVELOPMENT
PO9	Hariri, Fadi PhD program	NF-KBACTIVATION IN LYMPHOCYTES LEADS TO TRANSCRIPTIONAL STIMULATION OF THE EUKARYOTIC TRANSLATION INITIATION FACTOR EIF4E
PO10	Lisi, Veronique PhD program	IDENTIFICATION OF MICRORNA/TRAN SCRIPTION FACTOR AUTO-REGULA TORY LOOPS & THEIR ROLE IN PROLIFERATION & HEMATOPOIESIS
PO11	Methot, Stephen Masters program	CYTOPLASMIC RETENTION IS THE MAJOR FORCE RESTRICTING AID NUCLEAR ACCUMULATION & BIOLOGICAL ACTIVITY.
PO12	Neveu, Benjamin Masters program *(absent)*	IDENTIFICATION DE NOUVELLES CIBLES TRANSCRIPTIONNELLES DU FACTEUR DE TRANSCRIPTION ETV6
PO13	Nichol, Jessica PhD program *(absent)*	TARGETING PKCΔ -MEDIATED TOPOI SOMERASE IIB OVEREXPRESSION SUBVERTS THE DIFFERENTIATION BLOCK IN A RETINOIC ACID-RESIS TANT APL CELL LINE

PO14	Nielsen, Torsten PhD program *(absent)*	COMBINATION THERAPY WITH THE HI STONE DEACETYLASE INHIBITOR PANOBINOSTAT AND THE CD20 TAR GETING ANTIBODY RITUXIMAB IN DIF FUSE LARGE B-CELL LYMPHOMA.
PO15	Orthwein, Alexandre PhD program	REGULATION OF AID STABILITY BY THE DNAJA1-HSP90 MOLECULAR CHAPER ONING PATHWAY SUGGESTS POSSI BLE LEUKEMIA/LYMPHOMA THERAPY.
PO16	Pécheux, Lucie Masters program	EVALUATION OF HISTONE DEMETHYLASES EXPRESSION IN PRIMITIVE HEMATOPOIETIC STEM CELLS AND PEDIATRIC LEUKEMIA SAMPLES
PO17	Rashkovan, Marissa Masters program	THE MYC-INTERACTING ZINC-FIN GER PROTEIN-1 (MIZ-1) CONTROLS NOTCH1 INDUCED T-CELL LYMPHOMAGENESIS IN MICE
PO18	Siddiqui, Nadeem Post PhD program	STRUCTURAL CHARACTERIZATION OF DEPTOR: AN ENDOGENOUS MTOR INHIBITOR FREQUENTLY OVEREX PRESSED IN MULTIPLE MYELOMA CELLS
PO19	Simon, Camille PhD program	A KEY ROLE FOR EZH2 AND ASSOCIATED GENES IN MOUSE AND HUMAN ADULT T CELL ACUTE LEUKEMIA
PO20	Spinella, Jean-Francois PhD program *(absent)*	THE GENOMIC LANDSCAPE OF CHILDHOOD PRE-B ACUTE LYMPHOBLASTIC LEUKEMIA
PO21	Vasquez, Gabriela PhD program	THE TWO ISOFORMS OF EUKARYOTIC INITIATION FACTOR (EIF) 4A, DISPLAY DIFFERENT FUNCTIONS AT THE CELLULAR LEVEL

PO22	Vincent, Krystel PhD program	DISCOVERING OPTIMAL TARGETS FOR ADOPTIVE T-CELL CANCER IMMUNOTHERAPY OF LEUKEMIA
PO23	Wang, Xue Qing David Masters program	FUNCTION OF NON-CODING RNAS IN HOX GENE CLUSTERS OF MIXED LINEAGE LEUKEMIAS

PO1

Title: Multiple Factors Converge on mapk Splicing in Drosophila

Authors: Dariel Ashton-Beaucage, Christian Udell, Hugo Lavoie, Caroline Baril, Martin Lefrançois, Anne-Sophie Guenier, Jean Duchaine, Daniel Lamarre, Patrick Gendron, Sébastien Lemieux, Marc Therrien

Affiliation: Marc Therrien, IRIC, Université de Montréal.

Keywords: RAS; MAPK; RNAi screen; splicing; Drosophila

Background information: The RAS/MAPK signalling pathway is a central element of the communication machinery that relays proliferation and differentiation signals from the cell's surface to the nucleus. It plays key roles in many developmental processes and in many aspects of normal adult life. Importantly, oncogene driven activation of MAPK signalling is tightly associated to the development and progression of cancer.

Purpose of the study: Our aim is to identify the network of cellular components that is involved in RAS/MAPK signalling.

Methods: In order to better understand the regulatory network underlying the RAS/MAPK signaling module, we conducted an unbiased functional genomic screen. We used a genome-wide RNAi library to identify genes that modulate MAPK signalling in Drosophila S2 cells.

Results: This experiment led to the identification of mapk mRNA processing factors, including splicing factors and the exon junction complex (EJC) as pathway regulators. Further analysis revealed that these components could be positioned downstream of the MAPKK, MEK and were subsequently found to impact mapk expression itself. In particular, the EJC, a complex traditionally associated to post-splicing regulatory events, was unexpectedly found to act on mapk splicing. Other factors identified in the screen were also found to impact mapk splicing. However, the specific exon skipping events associated with these factors differed from those observed for the EJC.

Conclusion: We propose that the particular gene structure of mapk – the presence of long introns and weak splice sites – makes this Drosophila gene a choice target for splicing modulation. This would explain why this specific point of the RAS/MAPK pathway requires regulatory input from multiple splicing factors.

PO2

Title: C-terminal domain of MEIS1 converts PKNOX1 into a HOXA9-collaborating oncoprotein

Author: Richard Bisaillon

Affiliation: Dr. Guy Sauvageau, Laboratory of Molecular Genetics of Stem Cells, Institute for Research in Immunology and Cancer, Montréal.

Keywords: Transcription, myeloid leukemia, homeoproteins

Background: There is a compelling body of evidence implicating the HOX transcription factors and their co-factors PBX and MEIS/PKNOX in leukemogenesis. In particular, deregulated expression of HOXA9 has been detected in a large proportion of human acute myeloid (AML) and lymphoid leukemias (ALL) and is associated with poor prognosis for refractory AML. A significant proportion of these leukemias, especially those harbouring MLL rearrangements, also over-express MEIS1, indicating that activation of MEIS1 may represent a key collaborating genetic event in leukemia development. Pknox1 over-expression, in contrast, failed to accelerate the occurrence of Hox9-induced AML, supporting the possibility that PKNOX1 exerts tumor suppressive activity.

Purpose of the study: Two domains, the HD and HMA/HMB, have been highly conserved during evolution of MEIS and PKNOX families. In contrast, their C-termini (CTD), are highly divergent. We postulated that the proposed opposing roles of MEIS1 and PKNOX1 in leukemogenesis map to their distinct carboxy termini.

Methods: To test this hypothesis we generated fusion PKNOX1 proteins comprising MEIS1 CTD, in the presence or absence of the PKNOX1 CTD, and tested their transforming functions in the in vivo leukemia-initiating assays. To further gain mechanistic insights into the leukemia development process, we used microarrays to analyze the global gene expression profiles of primary bone marrow cell populations engineered to co-overexpress Hoxa9 plus Meis1, or Pknox1, or the Pknox1-MC mutant.

Results: In this report we demonstrate that a chimeric protein generated by fusion of the MEIS1 C-terminal region encompassing the transactivating domain with the full length PKNOX1 (PKNOX1-MC) acquired the ability to accelerate the onset of Hoxa9-induced leukemia in mouse bone marrow transduction/transplantation model. We further show that in the absence of PKNOX1 CTD this function can be provided for by the transactivating domain of VP-16. We also show that the transforming ability conferred to PKNOX1 by MEIS1 CTD correlates with a distinct gene expression profile associated with wild type Meis1, but not Pknox1 over-expression. More specifically, primary bone marrow cells transduced with Hoxa9 + Meis1, or Hoxa9 + Pknox1-MC revealed perturbations in overlapping functional gene subsets implicated in DNA packaging, chromosome organization and in cell cycle regulation.

Conclusion: We show that activation of oncogenic pathways comprising the Meis1 target genes can be attributed to the transactivating function exhibited by the MEIS1 CTD. PKNOX1, however, has no identifiable transactivation domain and shares the target sequences with MEIS, suggesting that PKNOX1/PBX and MEIS1/PBX complexes could be interchangeable at some regulatory sequences. PKNOX1 could thus suppress the oncogenic pathways by preventing the MEIS1-dependent recruitment of transcriptional co-activators and/or co-repressors to the target loci. Supporting this possibility we show that addition of MEIS1 CTD to full length PKNOX1 is sufficient for activation of Meis1-associated oncogenic pathways, and that MEIS1 and PKNOX1 differentially regulate the expression of a subset of genes involved in cellular proliferation.

PO3

Title: Involvement of TGF-β in the creation of an immunosuppressive microenvironment during leukemia

Authors: Cédric Carli, Catherine Jean, Louis-Philippe Caron, Amina Dahmani, Martin Giroux and Jean-Sébastien Delisle

Affiliation: Department of Médecine, CR-HMR, Université de Montréal.

Keywords: TGF-β, leukemic microenvironment, anti-leukemia immune response, immunosuppression, immunotherapy

Background: Allogeneic hematopoietic cell transplantation (AHCT) allows the transfer of immune cells from a healthy donor to eradicate tumor cells in leukemic patients. However, tumors develop a milieu that fosters their development and counteract the total efficacy of immune system through different mechanisms. Notably, neoplastic cells secrete mediators like Transforming growth factor-beta (TGF-β), a potent growth suppressor and immunosuppressive cytokine that has the capacity to inhibit the anti-tumor immune response in a wide variety of cancers. While TGF-β has been found at high levels in several hematopoietic malignancies, its impact on the generation of a leukemic immunosuppressive microenvironment has never been extensively evaluated.

Purpose: We aim to characterize the role of TGF-β in shaping the leukemic microenvironment in order to devise better immunotherapies.

Methods: TGF-β producing EL4 murine leukemia cells were inoculated in the flank of syngeneic mice with or without the blocking antibody anti-TGF-β. 21 days after, the tumor, spleen and bone marrow cellular composition was evaluated by flow cytometry and a broad range of cytokines liberated in tumor milieu was quantified.

Results: In our in vivo model, EL4-derived tumor elaborated a complex microenvironment and attracted a wide variety of leukemia infiltrating leukocytes (LILs). About 10% of tumor cellularity was composed by immune cells, including a large proportion of T lymphocytes and myeloid cells (mainly neutrophils and monocytes). When mice were treated with anti-TGF-β, the microenvironment was significantly modified with an increase in the proportion of effector T lymphocytes and more surprisingly of myeloid cells, unveiling a potential role for these cell types in leukemia specific immunity. Moreover, cytokines involved in migration and activation of leucocytes, namely IL-2, GM-CSF and MIP-1α secretion in was significantly induced compared to control.

Conclusion: Our data suggest that TGF-β blockade alters the leukemic microenvironment in a way that is relevant to the efficient targeting of leukemia by immune cells. Further studies will confirm the impact of TGF-β on the leukemic milieu including LILs, stromal cells and angiogenesis and how this immunosuppressive cytokine impedes immunotherapy. Leukemia immunotherapeutic approaches will benefit from the identification and targeting of immunosuppressive networks within the tumor microenvironment.

PO4

Title: The role of translesion synthesis polymerase REV1 in generation of point mutations in mammalian cells

Author: Sayeh Davoudi (1,2), Lam Leduy (1), Laura Hulea (1,2) , Zubaidah Mohamed-Ramdzan (1), Alain Nepveu (1,2,3)

Afilliations: Goodman Cancer Research Center (1) , Departments of Biochemistry (2) and Oncology (3), McGill University.

Keywords: Mutations, REV1, BCR-ABL

Background: Chronic myelogenous leukemia (CML) is caused by a chromosomal translocation that generates the so-called Philadelphia chromosome and fuses the BCR and ABL genes (1, 2). The tumorigenic potential of BCR-ABL protein requires a functional tyrosine kinase domain (3). Imatinib mesylate (IM) has become the treatment of choice for patients with chronic-phase CML who are not candidates for immediate stem cell transplantation (SCT). Despite amazing success, treatment with IM fails in 25% of cases due to primary refractoriness (primary resistance) or loss of response (LOR) after initially achieving required response criteria (secondary resistance). Resistance to imatinib most often results from point mutations in the BCR-ABL catalytic domain that prevent binding of the IM inhibitor (6, 7).

Purpose: 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.
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. 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.

PO5 *(absent)*

Title: The effect of organic solvents and CYP1A1, CYP2E1, GSTM1 polymorphisms on the development of acute lymphoblastic leukemia in Quebec children

Author: Kimberley Doucette

Affiliation: Dr. Claire Infante-Rivard, Department of Epidemiology, Biostatistics and Occupational Health.

Keywords: acute lymphoblastic leukemia, organic solvents, household solvents, xenobiotic metabolizing genes, gene-environment interaction

Background: Childhood acute lymphoblastic leukemia (ALL) is a complex disease whose etiology remains largely unknown. Both genetic and environmental factors are believed to be involved in leukemogenesis. It has long been suspected that organic solvents are carcinogens. They are common in the workplace and are potentially important sources of exposure in mothers during various time periods: preconception, pregnancy and postnatal. These time windows are vital for the developing fetus and exposures to carcinogens through the placenta or breast milk could lead to DNA damage. In addition, variants in xenobiotic metabolizing genes that biotransform various chemicals entering the body, in particular CYP (cytochrome P450) and GST (glutathione S-transferase) genes, have equally been linked to the development of ALL.

Purpose of the study: Our goal was to assess whether CYP and GST genes affect the biotransformation of chemicals such as organic solvents in the fetus or infant, leading to increased DNA damage and potentially cancer.

Methods: I analyzed the effects of maternal occupational exposure to organic solvents during pregnancy and breastfeeding on the risk of developing ALL in the offspring. The effects of organic solvents from household activities were also investigated in breastfeeding mothers during the postnatal period. In addition, I analyzed the joint effects of case genetic variants in certain likely functional xenobiotic metabolizing genes (CYP1A1, CYP2E1 and GSTM1) with organic solvent exposures. The data was taken from a large population based case-control with 790 cases and 790 controls recruited from Quebec, Canada. It was analyzed using logistic regression and Poisson log-linear models based on case-control, case-only and case parent-trio designs.

Results: Significant main effects were found between case GSTM1 null and CYP1A1 *4 variants and ALL. Additionally, individuals with one copy of the CYP1A1 *2A variant and GSTM1 null had a significant odds ratio of developing ALL as compared to an individual with neither. Offspring with the GSTM1 null variant whose mothers were occupationally exposed to aliphatic alcohols and aliphatic ketones, specific chemical families of organic solvents, during pregnancy had a lower risk of developing ALL than carriers of the wild type carriers. Among mothers who breastfed, there was evidence to suggest that the GSTM1 null and CYP1A1 *2A variants modified the effect of solvent exposure from furniture stripping, and likewise for the CYP2E1 *5 variant with certain activities involving exposure to electronics. The CYP1A1 *2A variant also appeared to significantly modify the effect of latex and/or acrylic paint exposures in a breastfeeding mother on the risk of ALL.

Discussion: Although the study had limited power to uncover statistically significant interactions, the results suggest a role on the incidence of childhood ALL for gene variants involved in the metabolism of carcinogens in the presence of environmental prenatal or breastfeeding exposure to organic solvents.

PO6

Title: Comparison of the overexpression of HOXA4 and HOXB4 in hematopoietic stem and progenitor cells in vitro and in vivo

Author: Marilaine Fournier

Affiliation : Laboratoire du Dr. Janet Bijl, Département de médecine de l'université de Montréal, Centre de recherche de l'Hôpital Maisonneuve-Rosemont.

Keywords: Hematopoietic stem cell (HSC), HOXA4, HOXB4, HSC expansion, Bone marrow transplantation

Background: HOX genes are known for their involvement in self-renewal of hematopoietic stem cells (HSC). Enforced expression of HOXB4 leads to HSC expansion in vitro and in vivo without leukemia development. We have recently shown that overexpression of its paralog HOXA4 also resulted in an increase of HSC and myeloid progenitors in vitro. HOXA4+ HSC are fully functional and reconstitute mouse chimeras with normal ratios of mature cells in the peripheral blood. Although HOXA4 and HOXB4 both have the ability to expand HSC, inferior engraftment of HSC mutant for Hoxa4 has been observed contrarily to HSC lacking Hoxb4. Expression studies also showed a 10-fold higher expression of Hoxa4 compared to Hoxb4 in HSC enriched fetal liver (E14.5) fractions at which development stage HSC are undergoing their principal expansion. These studies suggest that under physiological conditions HOXA4 may be more important in HSC expansion than HOXB4.

Purpose of the study: The aim of the study is to compare the expansion potential of HOXA4 and HOXB4 on BM cells in a context of bone marrow (BM) transplantation.

Methods: To compare the expansion potential of HOXA4 and HOXB4 on BM cells in vitro, individual and competitive cultures of primary BM cells overexpressing HOXA4 and/or HOXB4 were initiated. Colony forming cell (CFC) assays and flow cytometry analysis were performed to assess the frequency of myeloid progenitors and the phenotype of the cultures.

Results: While BM cells overexpressing HOXA4 or HOXB4 grew better than control cells, (up to 100 times higher within 21 days), no difference in growth or outcompetition was observed between single and within competition cultures, respectively. Colony forming cell assays showed comparable numbers of HOXA4 and HOXB4 myeloid progenitors in these cultures over time. Additionally, flow cytometry analysis revealed equally sized HSC-enriched LIN-c-kit+Sca-1+ (SKL) CD48- subpopulation in HOXA4+ and HOXB4+ cultures. These results indicate that HOXA4 and HOXB4 have a similar effect on expansion of BM cells in vitro. To examine the repopulation abilities of HSC overexpressing HOXA4 or HOXB4 in vivo, low doses of 10 000 HOXA4+, HOXB4+ or control BM cells were transplanted in lethally irradiated recipients in competition with wild type BM cells. Myeloid and lymphoid engraftment by HOXA4 or control cells in the periphery was comparable in recipients 8 weeks post transplantation. Interestingly, HOXB4 chimeras showed an increase in myeloid cells (Mac-1+) in the periphery at the expense of lymphoid cells (CD3+, $p<0.05$). Long-term engraftment is currently under investigation.

Conclusion: These results indicate that HOXA4 may be more suitable than HOXB4 for a normal reconstitution following BM transplantation.

PO7

Title: Diminished Interleukine-7 Levels and Low Dendritic Cell Counts Contribute to Constrain Homesotatic Peripheral Expansion of CD4+ T Cells During Graft-Versus-Host Disease

Authors: Simon-David Gauthier, Dominique Leboeuf and Martin Guimond. Hôpital Maisonneuve-Rosemont Research Center, Université de Montréal, Montréal, Canada.

Keywords: Bone marrow transplant, Graft-versus-host disease, Immune reconstitution, CD4+ T cells

Background Information: Allogeneic bone marrow transplant (BMT) is an effective treatment for numerous types of haematological malignancies. However, graft-versus-host disease (GVHD) is a serious complication and its adverse effect on T cell regeneration greatly exaggerates the immunodeficiency normally associated with BMT rendering patients at higher risk to common and opportunistic infections, tumor recurrence or second malignant neoplasm. Regeneration of lymphocytes can occur via a thymic dependent pathway and thymic independent pathway termed “homeostatic peripheral expansion” (HPE). In most clinical settings of BMT and GVHD, thymopoiesis is loss and HPE is the unique pathway that can restore T-cell counts after lymphodepletion. However, while HPE efficiently reconstitutes CD8+ T lymphocytes, CD4+ reconstitution is typically inefficient with deficit often persisting for several years.

Hypothesis: Given that elevated systemic IL-7 levels found in lymphopenic host has been proposed to signal on DCs and diminish CD4 HPE, we hypothesized that removal of IL-7 signaling in DCs could improve immune reconstitution of CD4+ T cells during GVHD.

Methods: To study the impact of GVHD on thymic independent pathway of immune reconstitution, we used the mouse model B6◊B6D2F1 and measured the homeostatic peripheral expansion (HPE) of anti-HY transgenic CD4+ T cells in GVHD hosts. Since IL-7Rα-/- dendritic cells support efficiently HPE of naïve CD4+ T cells in lymphopenic animals, we transplanted B6D2F1 recipients with BM cells from B6IL-7Rα-/- mice and GVHD was induced by adding 1x106 B6 T cells.

Results: As predicted, non GVHD hosts supported efficiently HPE of anti-HY CD4+ T cells. In contrast, GVHD hosts could not support CD4 HPE and this occurred despite lack of IL7 signaling in DCs. Surprisingly, GVHD was associated with a severe depletion of all DC subsets. The absence of antigen presenting cells was in part due to a myelosuppression affecting DC production from the bone marrow that was further compounded by their allorecognition by GVHD T cells. Interestingly, treatment with Flt3-ligand (FL) could significantly increase the number of DCs, yet it was insufficient to support CD4 HPE. However, combining FL and IL-7 treatments was successful to significantly restore HPE of CD4+ T cells.

Conclusion: Thus far, our data support a model wherein GVHD insults to the bone marrow microenvironment constrains FL and IL-7 productions which are both required for the restoration of the peripheral niche that supports CD4 HPE.

PO8

Title: Exploring the role of SCL, LMO1 and NOTCH1 oncogenes in pre-leukemic T cell development

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Keywords: NOTCH1, SCL, LMO, self-renewal, pre-leukemic stem cell.

Background information: Self-renewal and multipotency are distinctive properties of normal hematopoietic stem cells (HSCs) that are controlled in part by SCL, a basic helix-loop-helix (bHLH) transcription factor and LMO1/2, a LIM domain protein that associates with SCL. HSC-derived progenitors that settle in the thymus are devoid of these stem-cell properties and progress through several stages of differentiation (ETP/DN1, DN2-4, DP) before giving rise to CD4+ or CD8+ immune-competent T cells. The NOTCH1 pathway is indispensable for T-cell development in the thymus and controls the T versus B cell fate. More than half of pediatric T cell acute lymphoblastic leukemia (T-ALL) harbour NOTCH1 mutations leading to the constitutive activation of the pathway. Furthermore, ectopic expression of SCL and LMO1/2 is found in almost 25% of T-ALL. Remarkably, T-ALL samples exhibit complex genetic alterations and it is not clear which ones are driver or passenger oncogenic events. We took advantage of a transgenic mouse model that closely reproduces human T-ALL to show that SCL, LMO1 and NOTCH1 are sufficient to transform normal thymocytes and induce leukemia.

Purpose of the study: Here, our goal is to define the contribution of each oncogene to the process of thymocyte transformation before leukemia onset. Precisely, we want to determine whether SCL, LMO1 and NOTCH1 can induce stem cell-like properties to pre-leukemic thymocytes.

Methods: To this end, we transplanted pre-leukemic thymocytes into sublethally irradiated isogenic mice to explore their self-renewal capacities. Thymocytes were also cultured in contact with OP9 stromal cells encoding the NOTCH ligand / Delta-like1 (OP9-DL1) allowing in vitro T cell differentiation. Gene expression was assessed by global gene profiling and qRT-PCR.

Results: Our results indicate that SCL-LMO1 oncogenes induce a stem cell gene signature to pre-leukemic thymocytes and confer aberrant self-renewal potential to these cells. Unexpectedly, we observe that SCL-LMO1 confer aberrant B cell lineage potential to thymocytes in vivo and in vitro. In particular, SCL-LMO1 allow for B-cell development under conditions that should give only T-cells, i.e. in response to Delta-NOTCH1 stimulation, correlating with decreased expression of NOTCH target genes in DN1-DN3 thymocytes in vivo. In contrast, the NOTCH1 oncogene does not confer self-renewal properties to thymocytes. Rather, NOTCH1 promotes the expansion of self-renewing SCL-LMO1 thymocytes and inhibits their B cell lineage potential. Finally, we show that SCL-LMO1 confers aberrant self-renewal to DN1 and DN3 thymocytes whereas increased B lineage potential is observed in a subset of DN1 cells only.

Conclusion: Our observations are consistent with the view that the SCL-LMO1 oncogenic transcription factors reprogram DN1-DN3 thymocytes to acquire self-renewal potential, thereby establishing a pre-leukemic state. Furthermore, SCL-LMO1 inhibit the NOTCH pathway and enhance the bi-lineage potential of DN1 thymocytes. Finally, NOTCH1 activation provides a strong signal that collaborates with the SCL-LMO1 oncogenes to induce T-ALL by favoring the clonal expansion of pre-leukemic stem cells.

PO9

Title: NF-κBactivation in lymphocytes leads to transcriptional stimulation of the eukaryotic translation initiation factor eIF4E

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Keywords: Transcription, NF-κB, eIF4E, Leukemia and Lymphoma

Background Infromation: The eukaryotic translation initiation factor, eIF4E, plays a pivotal role in regulating patterns of gene expression by functioning at two levels: mRNA export and translation, both of which are dependent on 7-methyl guanosine cap binding. It is overexpressed in 30% of human cancers including breast, head and neck carcinomas as well as in Hodgkin and non-Hodgkin lymphomas and M4/M5 subtypes of acute myeloid leukemia (AML). Studies have demonstrated that eIF4E activity is regulated at multiple levels including: post-translational modification, mRNA stability and multiple protein interactions. In contrast, much less is known about the transcriptional regulation of eIF4E. Over the last 15 years, cMyc has been the only factor implicated in the transcriptional control of eIF4E, despite the fact that eIF4E is still produced in Myc null fibroblasts.

Purpose of the study: Our previous studies in primary leukemia specimens (M5 AML) suggested a strong link between the oncogenic transcription factor NF-κB and eIF4E. We aimed to investigate whether eIF4E is a direct transcriptional target of NF-κB.

Methods: Inhibition of NF-κB activity through the introduction of the inhibitor IkB-super repressor (IkB-SR) in primary CD34+ M5 AML specimens, characterized by constitutive NF-κB activity, resulted in a substantial reduction in eIF4E transcript and protein levels. We therefore investigated the possibility of a regulatory role for NF-κB proteins vis a vis the transcriptional control of the eIF4E promoter. The eIF4E promoter harbors four putative NF-κB binding sites as determined from previous reports and through recent MatInspector analysis. We have used electrophoretic mobility shift assays (EMSA) and chromatin immunoprecipitation (ChIP) to validate a physical interaction between NF-κB proteins and the eIF4E promoter. Additionally, eIF4E transcript inducibility was monitored in a variety of cell lines as well as primary specimen through QRT-PCR in response to NF-κB activating stimuli.

Results: In primary human blood cells, fibroblasts and lymphoma cell lines, eIF4E protein and mRNA levels were induced by PMA as early as two hours post-stimulation. Upregulation of eIF4E was completely reversed by pharmacological inhibition of NF-κB activity. We identified four putative κB sites in the eIF4E promoter region able to bind p65 and cRel subunits in PMA stimulated B cells. Analysis of the endogenous promoter demonstrated recruitment of p65 and cRel to two of the four κB sites as early as one hour post-PMA treatment. Transcriptional activation was evidenced by recruitment of p300 and phosphorylated Pol II. Our results provide a direct link between the NF-κB pathway and eIF4E expression levels.

Conclusion: This study is the first to explore the mechanistic link between NF-κB and eIF4E activity. Understanding this level of regulation within the context of patient specimens is important for the development of novel therapeutic strategies, such as combining ribavirin treatment with specific NF-κB inhibitors in leukemia patients, in an attempt to more effectively target eIF4E and NF-κB networks.

PO10

Title: Identification of microRNA/Transcription Factor Auto-Regulatory Loops and their Role in Proliferation and Hematopoiesis

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Keywords: microRNA, transcription factor, computational approach

Background: MicroRNAs (miRNAs) have recently been recognized as important actors of the mechanisms leading to cancer (1), and were shown involved in various regulation networks and in particular in auto-regulatory loops with transcription factors (TFs) (2). It is thus reasonable to hypothesize that modifications in either miRNA or TF expression levels can result in a cascade of misregulations that could ultimately cause cancer.

Purpose of the study: To identify and measure the abundance of such loops, we devised an algorithm that predicts these loops and validated some of the results experimentally. Doing so, we uncover new roles for miR-223 and miR-363 in hematopoiesis.

Methods: Our algorithm is based on TargetScan and a customized version of the MatInspector algorithm applied to position weight matrices from Transfac and Jaspar (3-6). Applied to the human and mouse genomes, our algorithm predicts over 700 miRNA/TF auto-regulation loops involving 130 miRNAs and 182 TFs**.**

Results: From the predictions of our algorithm, we have validated the following loops: LMO2/miR-223, LMO2/miR-363, CEBPα/miR-155, CEBPα/miR-212, and GATA2/miR-489. Since LMO2 has been shown an important factor in hematopoiesis and is a major cause of T-cell leukemia (7), we further investigated the loops involving LMO2. We measured their effect on primary cell proliferation and demonstrated their importance in an in vivo context. We transplanted miR-223 or miR-363 infected cells in irradiated mice and measured the hematopoietic reconstitution showing important consequences of the loops in hematopoiesis.

Conclusions/Significance: This project is among the first genome-wide attempts at predicting and validating miRNA/TF auto-regulatory loops. Our approach reveals the auto-regulatory loops of miRNAs but provide only a glimpse at their secondary effects; that is the effects of their target, which will likely prove important in the future. Furthermore, the implications of the auto-regulatory loops identified here go far beyond the direct implication of the factors involved in these loops and deserve additional studying.

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PO11

Title: Cytoplasmic retention is the major force restricting AID nuclear accumulation and biological activity

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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 leukemias. 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. Our lab recently discovered AID CR, and it is still unclear how both NE and CR are implicated in restricting nuclear accumulation of AID.

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), in human B cell lines and followed endogenous AID localization by immunofluorescence. To further understand its importance, we checked whether CR is evolutionarily conserved, like NE, by expressing AID homologs tagged with GFP in different cell lines from different species. Finally, in order to investigate the biological importance of AID CR, we tested the various activities of AID mutants deficient in CR. We tested enzymatic activity by determining mutagenic rates in transformed E. coli, class-switching activity by reconstituting AID-/- B cells and monitoring switched IgG1 levels, and somatic hypermutation activity by reconstituting AID-/- DT40 B cells and monitoring the loss of IgM due to AID 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, further promoting its importance in regulating AID localization. Interestingly, we observed that mouse AID did not respond strongly to leptomycin B. This was confirmed in mouse B cells, and is due to two residues in the C-terminus of mouse AID that increase hydrophobicity. Finally, we determined that though mutations affecting AID CR tend to cause a modest reduction in enzymatic and class switching activity, they cause up to 3-fold higher somatic hypermutation activity, confirming the importance of CR in regulating AID biological activity.

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 somatic hypermutation. Future studies will look to confirm the biological importance of CR using knock-in cells and mice, as well as to elucidate the mechanism by which AID is retained in the cytoplasm.

PO12 *(absent)*

Titre: identification de nouvelles cibles transcriptionnelles du facteur de transcription ETV6

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Mots-clés: Leucémie, Transcription, Régulation, ETV6

Introduction: La leucémie lymphoblastique aigüe (LLA) est la forme la plus fréquente de cancer pédiatrique, comptant pour environ le tiers des cas. La translocation t(12;21) est observée dans environ 26% des cas de LLA de l'enfant, , ce qui en fait l'aberration génétique la plus fréquemment retrouvée dans ce type de leucémie. L'expression de la chimère résultante, ETV6-AML1, n'est pas suffisante pour initier la leucémogenèse, suggérant que des mutations supplémentaires sont requises afin de déclencher ce processus. Or, il a été observé que dans environ 75% des cas de LLA ayant la t(12;21), il y a également perte de l'allèle résiduel d'ETV6. L'inactivation complète d'ETV6 suite à ce second incident serait l'événement déclencheur de la maladie.

But de l'étude: Comme ETV6 est un répresseur de la transcription, il est d'intérêt d'identifier ses cibles transcriptionnelles pour comprendre comment la surexpression de celles-ci suite à la perte de l'expression d'ETV6 induirait le développement tumoral.

Méthodes: À l'aide d'analyses pan-génomiques de l'expression globales des gènes (expression microarrays), il est possible d'identifier les gènes dont l'expression est modifiée en fonction de la présence ou l'absence de la t(12;21) dans les patients LLA. Les cibles putatives d'ETV6 ainsi identifiées sont ensuite validées par qRT-PCR dans une cohorte de patients. Finalement, des expériences de gène rapporteur dans trois lignées cellulaires différentes et d'immunoprécipitation de la chromatine (ChIP) sont réalisées afin d'évaluer directement l'effet d'ETV6 sur les promoteurs ciblés.

Résultats: L'activité promotrice de ELK3, TERF2, CBFA2T3, RAG1, TCFL5, DPYSL2, FAM134B, RASA4 et SERINC5 est réduite en présence de l'expression d'ETV6 (expérience de co-transfection). Des expériences de ChIP confirment les résultats obtenus dans notre système d'essais de gène rapporteur. Nous avons ensuite mis en évidence l'importance des domaines fonctionnels d'ETV6 (i.e Pointed et ETS) sur la répression en utilisant des formes tronquées de la protéine. De façon intéressante, pour les promoteurs TCFL5 et TERF2, le domaine de liaison à l'ADN d'ETV6 (ETS) n'est pas nécessaire pour la répression, suggérant un mécanisme de répression alternatif.

Conclusion: L'identification de nouvelles cibles transcriptionnelles d'ETV6 et les études menées sur celle-ci ont permis de mettre en évidence la capacité d'ETV6 à réprimer la transcription par différents mécanismes, soit par une liaison directe à l'ADN via son domaine ETS, soit par son recrutement par d'autres facteurs de transcription.

PO13 *(absent)*

Title: Targeting PKCδ -mediated topoisomerase IIβ overexpression subverts the differentiation block in a retinoic acid-resistant APL cell line

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Keywords: acute promyelocytic leukemia, topoisomerase IIβ, PKCδ, differentiation

Background information: Acute promyelocytic leukemia (APL), is a subtype of acute myelogenous leukemia. At the genetic level, APL is characterized by a specific chromosomal rearrangement between the retinoic acid receptor alpha (RARA) and the promyelocytic leukemia (PML) genes. The resulting chimeric protein, PML-RARA, acts as a dominant negative inhibitor of normal retinoid receptor function. On the cellular level, the result is a block in granulocytic differentiation and an accumulation of myeloid progenitors. APL patients are treated with therapeutic doses of all-trans retinoic acid (RA), a vitamin A derivative that activates RARA and circumvents the differentiation block. Unfortunately, RA resistance develops in vivo, a phenomenon that can be modeled in vitro. Previously, we identified topoisomerase IIβ (TOP2B) as a novel mediator of RA-resistance in APL cell lines.

Purpose of the study: RA treatment leads to an increase of TOP2B protein levels. However mechanistic data on the causes of this upregulation have been lacking. We therefore sought to investigate the RA-mediated pathways leading to increased TOP2B expression.

Methods: In vitro derived RA-resistant cell lines are useful experimental models for the study of mechanisms of RA-resistance in APL. Our lab has previously isolated an RA-resistant subclone from the parental RA-sensitive cell line NB4, denoted NB4-MR2. Western blot analysis and quantitative real time PCR (qPCR) were used to measure protein and mRNA levels, respectively. Differentiation was assessed by morphological analysis, by immunofluoresence staining of PML nuclear bodies, by expression of the CD11c cell surface myeloid specific antigen, and by nitro-blue-tetrazolium (NBT) reduction.

Results: We speculated that RA may activate protein kinase C delta (PRKCD), leading to increased phosphorylation and stability of TOP2B. The activation of PRKCD by RA correlates with increased TOP2B levels in both NB4 and NB4-MR2 cell lines. Most strikingly, NB4-MR2 cells show substantially increased basal levels of activated PRKCD when compared to NB4. Pharmacological inhibition of PRKCD, targeted knockdown of PRKCD and expression of a dominant negative inhibitory form of PRKCD, all resulted in reduction of TOP2B levels. Additionally, co-treatment with the PRKCD inhibitor, Rottlerin, and RA resulted in the induction of an RA responsive reporter construct, as well as the endogenous RA target genes, CEBPE, CYP26A1 and RIG-I. Furthermore, the co-treatment overcame the differentiation block in the NB4-MR2 RA-resistant cells.

Conclusion: Cumulatively, our data suggest a model whereby inhibition of PRKCD decreases TOP2B protein levels, leading to a loss of TOP2B mediated repressive effects on RA-induced transcription and granulocytic differentiation. Our findings that RA-resistance in APL cells can be overcome by targeting both the PRKCD and RA pathways may provide a basis for the rational design of novel therapies for not only RA-resistant APL, but other more common leukemias that have increased TOP2B expression.

PO14 *(absent)*

Title: Combination therapy with the histone deacetylase inhibitor panobinostat and the CD20 targeting antibody rituximab in diffuse large B-cell lymphoma

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Keywords: Lymphoma, histone deacetylase inhibitor, rituximab, combination therapy.

Background information: Diffuse large B-cell lymphoma (DLBCL) is the most common sub-type of non-Hodgkin's lymphoma and although potentially curable with combination chemotherapy, it remains a therapeutic challenge in patients who do not respond. Recent work has demonstrated frequent mutations in histone modifying enzymes in DLBCL, particularly in histone acetyl transferase genes. We therefore, speculated that perturbations of epigenetic marks play a driving role in these cancers and that treatment with a histone deacetylase inhibitor (HDACi) might normalize acetylation levels and thus, alleviate the oncogenic potential of targeted cells.

Purpose of the study: Clinical trials have shown that HDACis as single agents are only effective in a minority of patients. We therefore, sought to find combination treatments to enhance the effect of HDACis. Herein, we describe our results investigating combination therapy with the HDACi panobinostat (LBH) and the CD20-targeting antibody rituximab in DLBCL.

Methods: Six DLBCL cell lines were treated with LBH and/or rituximab in vitro for 48 hours at concentrations tolerable in humans. Cell death and viability were measured by propidium iodide stain using flow cytometry, and manual cell counting using Trypan blue, respectively. No complement or immune effector cells were added to ensure that only direct signaling effects of rituximab binding to CD20 were measured. CD20 cell surface expression was measured by flow cytometry. Synergy of combination treatment was calculated using CalcuSyn software. Protein expression was assessed by western blot. In parallel with the in vitro studies, a phase II clinical trial investigating LBH+/- rituximab is being conducted. Correlative patient samples from lymph node biopsies taken pre- and post-treatment will be used to verify whether mechanisms of synergy observed in cell lines can be translated to human beings.

Results: In four out of six DLBCL cell lines, we see a synergistic increase in cell death with the combination of LBH and rituximab. We find that HDACi treatment does not increase cell surface expression of CD20, but rather CD20 expression remained unchanged or was reduced. Despite this, synergy between LBH and rituximab was still observed. Preliminary results suggest that activation of p38 MAPK is a possible mechanism of action underlying the synergistic effects of LBH and rituximab. A total of 16 patients have been enrolled in the clinical trial thus far. While it is too early to assess clinical benefit, we do achieve useful yields of good quality RNA and DNA from biopsies.

Conclusion: Here, we present data showing synergistic effects on cell death in DLBCL cell lines with the combination of LBH and rituximab. The anti-cancer effect of this novel therapeutic approach appears to depend on the presence of CD20 cell surface expression, but does not require an LBH-induced increase in CD20. We believe the combination of an HDACi with rituximab is a therapeutic strategy that merits further exploration in clinical trials and one such trial is currently ongoing at our institution. Mechanistically, our results point toward the activation of p38 MAPK as an important event in synergistic cell death induced by LBH and rituximab.

PO15

Title: Regulation of AID stability by the DnaJa1-HSP90 molecular chaperoning pathway suggests possible leukemia/lymphoma therapy

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Affiliation: Dr. Javier M. Di Noia, Laboratory of Genetic Diversity, IRCM.

Keywords: AID, molecular chaperone, antibody diversification, chronic myeloid leukemia, lymphoma.

Background information: Activation induced deaminase (AID) plays a central role in adaptive immunity. AID deaminates deoxycytidine to deoxyuridine in defined regions of the immunoglobulin (Ig) genes and initiates somatic hypermutation (SHM), and class switch recombination (CSR).While being essential for an effective immune response by underpinning antibody affinity maturation and isotype switching, the mutagenic activity of AID can also be oncogenic and causes genomic instability leading to the development of cancer. For instance, AID can be a disease progression factor by accelerating leukemia clonal evolution in Philadelphia chromosome-positive B cell acute lymphoblastic leukemia (ALL) and by mutating the BCR-ABL1 oncogene in chronic myelogenous leukemia (CML) and ALL, thus underpinning resistance to the therapeutic drug imatinib. Therefore, AID regulation, including the control of its protein level, is central to balancing effective immunity with cancer. Notably, AID shuttles between the cytoplasm and the nucleus but is predominantly cytoplasmic insteady-state, with cytoplasmic AID being much more stable than nuclear AID. These regulatory steps contribute to limit the exposure of the genome to AID but their mechanisms are unknown.

Purpose of the study: We aimed at identifying new cytoplasmic partners of AID that could regulate its stability and modulate its biological functions.

Method: Double-affinity purification of AID coupled to mass spectrometry identified several members of the HSP90 molecular chaperoning pathway as potential candidates.

Results: We demonstrated that HSP90 interacts and stabilizes AID in the cytoplasm, preventing its polyubiquitination and subsequent proteasomal degradation. Consequently, HSP90 inhibition results in a significant reduction of endogenous AID protein levels and correlates with a proportional reduction in AID-mediated antibody diversification. By the same means we can prevent the acquisition of mutations in BCR-ABL1. Furthermore, we showed that the first step in the HSP90 molecular chaperoning pathway is the interaction of AID with HSP70 and one specific HSP40 protein, DnaJa1. DnaJa1 is the limiting step in cytoplasmic AID stabilization. DnaJa1 farnesylation is required for DnaJa1-AID interaction. Modulation of DnaJa1 levels or its farnesylation impacts endogenous AID levels and antibody diversification. In vivo, DnaJa1-deficient mice display compromised response to immunization, resulting from reduced AID protein levels and isotype switching.

Conclusions: We conclude that AID is a novel HSP90 client that requires the HSP40 DnaJa1 cochaperone. This pathway stabilizes cytoplasmic AID and defines the physiological levels of AID. Albeit indirectly, HSP90 or farnesyltransferase inhibitors allow for the first pharmacological means to modulate AID and could be used for treating some lymphomas/leukemias or autoimmune diseases in which AID is a diseases progression factor.

PO16

Title: Evaluation of histone demethylases expression in primitive hematopoietic stem cells and pediatric leukemia samples

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

Background information: Chromosomal translocations involving the MLL (Mixed-Lineage-Leukemia) gene, frequently found in infant leukemia, are associated with a dismal prognosis. MLL is a histone H3 lysine 4 (H3K4) methyltransferase, an epigenetic regulator also essential for normal hematopoietic stem cell (HSC) function. Histone methylation can be erased by a new class of enzymes, the histone demethylases (HDM), and their role in regulation of gene expression and modulation of HSC fate is under investigation. Recent evidence also imparts a role for HDM in human disease, as exemplified by a Nup98-Jarid1a fusion oncoprotein in a case of pediatric leukemia. A preliminary HDM screen in mouse hematopoietic cells using a RNAi based strategy revealed one positive (Jhdm1f) and two negative (Jarid1b, Hif1an) regulators of HSC activity among this class of genes, with Jarid1b knockdown resulting in a gain of HSC activity and a net expansion of stem cells.

Purpose of the study: Our goal is to characterize the expression profiles of HDM in human hematopoietic cells in both the primitive and differentiated compartments, and to see if activation of some of these genes is deregulated in leukemia. To achieve this, human pediatric AML samples will be studied, in the presence and absence of MLL rearrangements (BCLQ).

Methods: A quantitative assessment of the expression of HDM will be performed by q-RT-PCR in subsets of HSC-enriched population from cord blood (CB) samples, in comparison to unselected (bulk) CB Cells.
Populations will be isolated from CB unit from Hema-Québec biobanking system, using an automated magnetic cell sorting strategy to deplete for differentiated cells (negative selection, Lin-) and to enrich the cells for HSC and progenitor content (positive selection, CD34+). Further purification will be performed by FACS to isolate CD34+Lin- CD38-CD45RA- cells.
To ascertain HSC frequencies of sorted cells, aliquots will be xenotransplanted at limiting dilutions in immunodeficient mice, and their output measured with human cell surface marker tracking in recipients.
For the leukemia cells, only homogenous samples with high leukemic blasts content will be selected for analyses.

Preliminary results and conclusions: Preliminary data suggest that HDM expression profiles will likely vary among human normal and leukemic stem cells, providing a tool for selecting gene candidates for further functional evaluation, either to target factors that could promote human HSC expansion, or inhibit leukemia maintenance.

PO17

Title: The Myc-Interacting Zinc-finger protein-1 (Miz-1) controls Notch1 induced T-cell lymphomagenesis in mice

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Keywords: Miz-1, Notch1, T-ALL

Background Information: The cell surface receptor and signaling molecule Notch1 is a key element in early T-cell differentiation. In leukemic cells of almost 50% of patients with acute T-cell lymphoblastic leukemia (T-ALL), the signaling through Notch1 is activated, in many cases by genetic mutations. It has been shown that the proto-oncogene c-Myc is one of the downstream effectors of the Notch pathway. The Myc-Interacting Zinc-finger protein-1 (Miz-1) can bind to 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. While Miz-1 itself functions as a transcriptional transactivator, it enables c-Myc to function as a transcriptional repressor notably of genes regulating cell cycle progression.

Purpose of the study: Our goal was to characterize the role of Miz-1 in the Notch1 pathway and to determine how this interaction affects Notch1-induced T cell lymphomagenesis.

Methods: In this study, we made use of Miz-1 mice lacking the POZ domain (Miz-1ΔPOZ), which have severe defects in T cell development. Crossing these mice with mice expressing a constitutively active form of Notch1 (NotchΔCT) allowed us assess the role of Miz-1 in Notch1-induced lymphomagenesis. Furthermore, we used a construct in which four Notch1 binding sites are fused to the luciferase gene to determine the effect of Miz-1 on Notch1 target gene expression in vitro.

Results: Our experiments indicate that Miz-1 deficiency results in increased expression of the Notch1 protein in T cell subsets, as well as up-regulation of Notch1 target genes such as Hes-1, Dtx1, CD25, but not c-Myc in pre T-cells. Moreover, we have evidence that Miz-1 represses a Notch1 responsive promoter, further underlining the possible role of Miz-1 in regulating expression of Notch1 target genes. We have also found that Miz-1 directly interacts with the Notch1 target gene repressor CBF1/Rbp-jk in vivo. Finally, we also observed that Notch-induced lymphomagenesis was accelerated in Miz-1ΔPOZ mice.

Conclusion: Our results show that Miz-1 plays an important role in the regulation of the Notch1 signalling pathway through interaction with the repressor CBF-1. Loss of a functional Miz-1 protein causes upregulation of Notch1 target genes and therefore accelerated Notch1-induced T cell lymphomagenesis.

PO18

Title: Structural characterization of DEPTOR: an endogenous mTOR inhibitor frequently overexpressed in multiple myeloma cells

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Keywords: Cancer, mTOR, mRNA Translation, Structural Biology

Background information: The mammalian target of rapamycin (mTOR) is a multi-domain protein which associates with different binding partners to form two major complexes: mTORC1 and mTORC2. mTORC1 is a key component of the PI3K/Akt signalling pathway which is frequently upregulated in cancer. Consequently, there is heightened interest to develop therapeutic strategies targeting aberrant mTORC1 signalling in cancer patients. 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. Although promising, a limitation of these inhibitors is their inability to completely suppress mTORC1 activity. Recently, DEPTOR was discovered as an endogenous mTOR binding protein that functions to inhibit both mTORC1 and mTORC2 activity. This finding provides a framework to study a novel mechanism to suppress mTOR activity.

Purpose of the study: To determine the three-dimensional structure of DEPTOR and biophysically characterize its interaction with mTOR.

Methods: Acquiring milligram quantities of recombinant protein is a pre-requisite for biophysical and high resolution structural studies. We have already cloned and isolated various fragments of mTOR. Full length DEPTOR and smaller fragments will be cloned and expressed in different hosts (E. coli, insect cells) to ensure maximum expression and solubility. Purification techniques, such as affinity chromatography and FPLC, will be used to isolate the pure protein. The three-dimensional structure will be determined by either NMR spectroscopy or X-ray crystallography. The thermodynamic and kinetic binding properties between the DEPTOR and mTOR binding domains will be analyzed using isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR).

Results: DEPTOR is a 409 residue protein and our bioinformatic analyses indicate that it consists of two 'DEP' domains and one 'PDZ' domain. DEP domains are typically ~80-90 amino acids and are commonly found in proteins that regulate G-protein signalling. Thus far, the role of the DEP domains in DEPTOR remains unknown. The PDZ domain is ~80-90 amino acids and also found in many proteins involved in signalling. In accordance with previous studies, we have found that the PDZ domain in DEPTOR directly interacts with central region of mTOR, adjacent to the Rapamycin binding domain.

Conclusions: Since we identified the minimal binding domains between mTOR and DEPTOR, we will continue their structural characterization by NMR spectroscopy and/or X-Ray crystallography. Ultimately, our studies should give additional mechanistic insight into regulating mTOR activity, and ultimately develop novel therapeutic approaches.

PO19

Title: A key role for ezh2 and associated genes in mouse and human adult 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 aims 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. In these studies, we exploited an Ezh2 conditional knock-out mouse model. Cre-mediated deletion was used to generate a mutated Ezh2Δ allele and abrogate production of Ezh2 protein specifically in adult bone marrow cells. Upon gene inactivation, we then monitored cancer development and characterized the immunophenotype and proliferation status of leukemic cells. We also evaluated histone methylation status as well as PRC2 complex integrity. To further address the role of PRC2 and associated genes in adult human T-ALL, we sequenced transcriptomes and exomes of 12 human adult T-ALL specimens using the Illumina HiSeq2000 platform and looked for the presence of nonsynonymous mutations, large and small insertions and/or deletions in PRC2 and other associated genes.

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Results: In this study we report high frequency of spontaneous □ T cell leukemia (T-ALL) occurrence in mice with bi-allelic deletion of Ezh2. All hemopoietic and non-hemopoietic organs analyzed showed massive infiltration of leukemic blasts expressing CD3 and variable levels of CD4 and CD8 surface markers. Interestingly, lymphoblasts were exclusively positive for the cell surface TCR□ marker and showed little residual H3K27 tri-methylation marks compared to controls. 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 also found mutations in previously characterized T-ALL associated genes including NOTCH1, PTEN, NRAS, TP53. Moreov r, we found several mutations/mis-regulations in chromatin-associated genes such as KDM6B, ARID1A, DNM2, SATB1 and IDH2

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, argues for a more generalized deregulation of this Polycomb protein complex and associated genes in T-ALL. More globally, our results suggest that several other regulators of chromatin state, not necessarily linked with PRC2, may be involved in the pathogenesis of T-ALL.

PO20 *(absent)*

Title: The genomic landscape of childhood pre-B acute lymphoblastic leukemia

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Keywords: Leukemia, Driver mutations, Exome, Next-generation sequencing

Background information: Precursor B-cell acute lymphoblastic leukemia (pre-B ALL) is the most frequent pediatric cancer. Increased understanding of the pathobiology of B-cell ALL has led to risk-targeted treatment regimens and increased survival rates. However, the causes and potential genetic contributions to this pediatric cancer remain largely unknown.

Purpose of the study: We are using next-generation sequencing technology to better understand the genomic landscape of pre-B ALL and to build a catalogue of variations involved in pediatric ALL onset and/or progression.

Methods: Using a unique quartet design that consists of matched tumor (at diagnosis) and normal (remission) samples, as well as DNA from both parents, we are able to incorporate parental sequence information to reduce sequencing errors and facilitate the identification of true variants within a given family. The goal of this project is to identify recurrent and private somatic mutations driving the leukemic process. Here, we report the deep-sequencing of the whole exomes of 30 childhood B-cell ALL quartets. Using Agilent's SureSelect target enrichment system and the ABI SOLiD sequencing platform, we generated over 4.0 Gb of sequence on average per sample with a mean coverage of 30X. Genome-wide genotyping (Illumina's Omni 2.5 array) was also performed on each normal-tumor pair for quality control and structural variant identification.

Results: Approximately 97% of the targeted region was covered ≥1X in each sample and on average, 80% of the targeted bases passed our thresholds for variant calling (≥ 5X coverage, MQV ≥ 20). Based on these criteria, about 25,000 SNVs were identified per normal sample. Somatic variants were identified through a custom workflow using publically available tools for variant annotation and data from the entire cohort to filter out germline variants and false-positive calls in the tumor samples. We report here the somatic mutation profiles of pre-B ALL genomes, and highlight genes/pathways with an increased burden of somatic loss of function variants.

Conclusion: Functional validation of these variants will lead to the identification of potential driver mutations that may play a direct role in leukemogenesis. Ultimately, this work will provide invaluable insights to understanding the genetic mechanisms underlying pediatric ALL, which could lead to the development of powerful clinical tools to improve detection, diagnosis and treatment of this childhood cancer.

PO21

Title: The Two Isoforms of Eukaryotic Initiation Factor (eIF) 4A, display different functions at the cellular level

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Affiliation: Dr. Jerry Pelletier, Biochemistry Department, McGill University.

Keywords: eIF4AI, eIF4AII, translation initiation

Background information: The PI3K/Akt/mTOR signaling pathway has been implicated in lymphoma survival. One of the important check points in this pathway is the regulation of protein synthesis. Translation initiation, considered to be the rate limiting step of protein synthesis, requires the activity of eukaryotic initiation factor (eIF) 4F, a complex consisting of eIF4E (the cap binding protein), eIF4G (a scaffolding protein) and eIF4A (an RNA helicase). eIF4F has been proposed as an anti-cancer target and our lab has identified a small molecule, silvestrol, as a result of a screen for inhibitors of eukaryotic translation initiation. This compound is able to chemosensitize Pten+/-Eμ-Myc lymphomas to the effects of doxorubicin, and further characterization revealed it as an inhibitor of eIF4A activity in vitro. eIF4A exists as two isoforms: eIF4AI and eIF4AII. They share 90-95% sequence identity and are considered to have the same activity in vitro. eIF4AI is the more abundant isoform, however eIF4AI/II display different patterns of expression within human tissues.

Purpose of the study: We aimed to validate eIF4A as an anti-cancer target, and evaluate its role in lymphomagenesis using an in vitro lymphoma model. Additionally, we investigated potential differences of the two eIF4A isoforms on the cellular level.

Methods: Silvestrol inhibits both eIF4A isoforms in vitro and can therefore not be used to perform studies to elucidate potential differences between eIF4AI and eIF4AII. We took advantage of RNAi to assess for the specific effects of the two eIF4A isoforms. We evaluated cell proliferation, translation rates (performing 35S-methionine labeling and polysome profiling), potential changes in cell cycle, as well as effects on cell death. Also, comparative immunoblotting was performed to quantify the amounts of eIF4AI/II present in cells. Finally, we undertook competition assays to corroborate the effects of eIF4AI/II suppression in a Tsc2+/- Eμ-myc lymphoma cell line (the Eu-Myc model is built based on the same genetic lesions found in human Burkitt's lymphoma).

Results: We found that suppression of eIF4AI but not eIF4AII leads to inhibition of cell growth and protein synthesis. Although generally less abundant than eIF4AI, eIF4AII protein levels increased to reach those of eIF4AI upon suppression of eIF4AI, suggesting a compensation mechanism However, eIF4AII was not able to compensate for eIF4AI activity, suggesting different roles for the two isoforms. The increase in eIF4AII protein levels was not a result of an increase in protein or RNA stability, but was due to a transcriptional response activated by inhibition of eIF4AI activity.
We saw a similar inhibitory effect on cell growth using RNAi against eIF4AI in Tsc2+/- Eμ-myc lymphoma cells in vitro, that is an increase in eIF4AII protein level, but no compensation for effects of inhibition of eIF4AI.

Conclusion: Our experiments indicate that eIF4AI and eIF4AII perform different roles in translation. We observed that suppression of eIF4AI had an inhibitory effect on the proliferation of Tsc2+/- Eμ-myc lymphomas.

PO22

Title: Discovering optimal targets for adoptive T-cell cancer immunotherapy of leukemia

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Keywords: Antigen Recognition, Cancer, T Lymphocyte function, Transplantation

Background information: Allogeneic hematopoietic cell transplantation is the most widely used form of adoptive T-cell cancer immunotherapy (ATCI) but has two major drawbacks. Indeed, donor lymphocytes are neither selected nor primed (activated) prior to transfer. This can lead to graft-versus-host disease in 60% of recipients and to tolerization of donor lymphocytes by tumor cells. These two caveats can be circumvented by the injection of primed antigen-specific CD8 T-cells targeted to either tumor associated antigens (TAAs) or to minor histocompatibility antigens (MiHAs). Several studies in humans have established the value of TAAs and MiHAs against solid tumors and leukemia. Importantly, the value of TAAs and MiHAs as target for antigen-specific cancer immunotherapy has never been assessed against the same tumor.

Purpose of the study: Our goal is to directly compare the therapeutic efficacy of T-cells targeted to TAAs vs. MiHAs. More specifically, we want to evaluate the in vivo anti-leukemic potential of MiHA- vs. TAA-primed CD8 T-cells and identify the mechanisms responsible for the differential anti-leukemia activity of antigen-specific T-cells.

Methods: We selected 8 antigens that are expressed on EL4 cells, a lymphoblastoma cell line derived from C57BL/6 mice and confirmed their immunogenicity by in vitro cytotoxicity assay. Our panel includes 4 MiHAs of known sequence (H3a, H4a, H7a and H13a), as well as 4 TAAs whose sequences were elucidated by mass spectrometry in collaboration with Dr. Pierre Thibault and his team. To identify the mechanisms responsible for the differential anti-leukemia activity of antigen-specific T-cells, we used MHC class I tetramers provided by the NIH Tetramer Core Facility to isolate and analyse CD8 T-cells specific for TAAs and MiHAs.

Results: Our results show that CD8 T-cells targeted to 4 out of the 6 tested MHC I tetramer are undetectable by flow cytometry. These results strongly suggest that some antigen-specific CD8 T-cells generated in vivo interact weakly with their MHC I/peptide complexes. To investigate this, we assessed the quality of peptide binding to the MHC I molecule for our 8 antigens. We found that the half-life of MHC I/peptide complexes at the cell surface and the binding affinities of the peptide for the MHC I molecule are similar for both categories of peptides. We also showed that the four antigens for which CD8 T-cells were undetectable by flow cytometry have binding affinities and half-life similar to the other antigens, suggesting that the quality of the MHC I/peptide interactions is not the underlying cause of decreased tetramer staining.

Conclusion: Our results suggest that some antigen-specific CD8 T-cells have a weak interaction with their MHC I/peptide complexes. It will be interesting to investigate whether and how other mechanisms of immunogenicity, such as TCR avidity and T-cell frequency, can influence the outcome of antigen-specific leukemia immunotherapy. Our studies will provide the first direct comparison of the anti-leukemic potential of MiHA- vs. TAA-primed CD8 T-cells. We believe that these crucial informations will serve a guide for selecting the best antigens for antigen-specific ATCI in humans.

PO23

Title: Function of non-coding RNAs in Hox gene clusters of Mixed Lineage Leukemias

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Affiliation: Dr. Josée Dostie, Department of Biochemistry, McGill.

Keywords: Non-coding RNA, Mixed Lineage Leukemia, Chromatin structure

Background Information: Mixed lineage leukemia is a disease marked by the fusion of the MLL methyltransferase gene and a secondary gene partner creating a chimeric protein. The production of this fusion protein leads to the aberrant activation of two key HoxA transcription factors (HoxA9 and HoxA10) that are tighly regulated during normal hematopoiesis. The overexpression of these genes during hematopoiesis is believed to block cells at their stage of differentiation and promote uncontrolled cell proliferation leading to leukemogenesis. The regulation of HoxA genes involve multiple molecular mechanisms many of which have yet to be fully elucidated. These include the transcription of non-coding RNAs (ncRNAs) and the three-dimensional structure of chromatin at the HoxA locus.

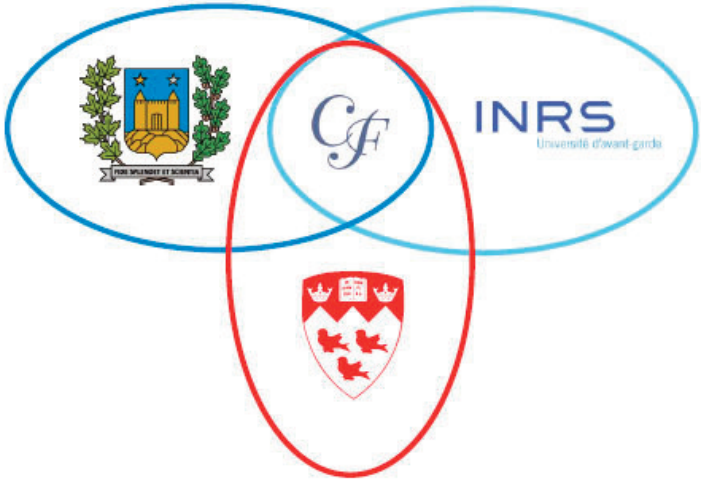
Purpose of study: This study focuses on the identification and elucidation of the function of non-coding RNAs and their relation to chromatin structure within the HoxA locus of mixed lineage leukemia.

Methods: We identified ncRNAs within a panel of MLL leukemia cell lines by using quantitative real-time PCR and high density tiling array. Northern blots were used to determine whether these transcripts contained alternatively spliced products. RNAi mediated knockdown of a target ncRNA (HOTAIRM1) was performed in the THP-1 leukemia cell line over the course of PMA mediated differentiation to elucidate its role in regulating HoxA gene expression. Chromosome conformation capture (3C) was performed in the HoxA cluster in undifferentiated and differentiated THP-1 cells to identify changes in chromatin contacts at key locations.

Results: Over the course of PMA mediated differentiation of the THP-1 cell line, we observe a switch in expression of ncRNAs at the 5'end of the cluster to a 3'end transcript named HOTAIRM1. Although the repression of the 5' transcripts correlate well with the reduction of nearby HoxA genes (HoxA9, A10, A11 and A13), the upregulation of HOTAIRM1 does not seem to follow any of its neighbor genes. However, using 3C technology, we observe a gain of contact between the HOTAIRM1 gene and an enhancer located between HoxA4 and HoxA5 after differentiation. Furthermore, RNAi mediated knockdown of HOTAIRM1 during differentiation shows a mild repression of several HoxA genes.

Conclusions: A number of ncRNAs are expressed from the HoxA gene cluster. From our THP-1 cell model, we observe that their expression pattern changes when cellular differentiation occurs. Our target of interest, HOTAIRM1, is induced during differentiation and seems to regulate the expression of several HoxA genes.

Notes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