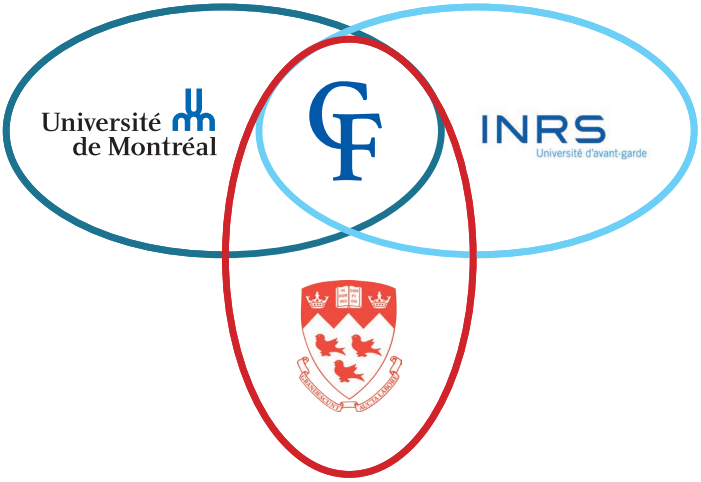
Cole Foundation
«Research Celebration Day»
May 5, 2017

11th Year



11ième Année

Journée
« Célébrons la recherche »
de la Fondation Cole
5 mai 2017

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 2017 Fellows, the Fellowship programme has supported more than 165 researchers in laboratories and hospitals situated in the Greater Montreal area through collaborations with l'Université de Montréal, McGill University, and INRS -Institut Armand-Frappier Research Centre. Over $10 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 establish- ment of the Jack Cole Chair in Pediatric Oncology and Hematology at McGill University.

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

The Cole Foundation

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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 2017, le programme a appuyé plus de 165 chercheurs répartis dans des laboratoires et des hôpitaux de l'agglomération montréalaise, à la faveur de collaborations avec l'Université de Montréal, l'Université McGill et le Centre INRS - Institut Armand-Frappier. Plus de dix 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 santés'intéressant aux soins et à la recherche en pédiatrie et à la découverte d'un traitement curatif pour la leucémie. Elle a été créée à la mémoire de sa fille, Penny Cole, emportée par la leucémie plus de dix ans avant. La Fondation appuie aussi des causes communautaires louables.

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

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

Program

9:30 - 10:30 AM	Round Table (new fellows) Salle M-425, Roger-Gaudry Building
10:00 - 11:00 AM	Poster Session Part 1
11:00 - 12:00 PM	Poster Session Part 2
12:00 - 12:45 PM	Lunch/Dîner
1:00 - 2:00 PM	Lecture Salle M-415, Roger-Gaudry Building
Welcome	**Dr Daniel Lajeunesse** Vice-Dean Basic Science Faculty of medicine, Université de Montréal
New Research - New Realities Presentations	**Dr Valérie Marcil** – Sainte-Justine University Hospital *Cardiometabolic complications in leukemia survivors* **Dr Alexandre Orthwein** – Lady Davis Institute *Influential role of DNA repair proteins in promoting B-cell lymphomas* **Dr Frédérick Antoine Mallette** – Hôpital Maisonneuve-Rosemont *Regulation of mTOR signaling by various mutations*
2:00 - 3:15 PM *Introduction*	**Dr Valérie Marcil** Assistant Professor-Researcher, Faculty of Medicine, Université de Montréal, CHU Sainte-Justine Research Center
Keynote Speaker	**Greg Armstrong,** MD, MSCE Member of the Department of Epidemiology and Cancer Control St. Jude Children's Research Hospital Memphis, Tennessee ***"The Lifetime Impact of Cancer and Cancer Therapy"***
3:15 - 3:30 PM	New fellows and announcement of prizes
3:30 PM	Reception

The 2017 - 2019 Cole Foundation Fellows

Les boursiers de recherche de la Fondation Cole 2017 – 2019

Université de Montréal

Elodie Da Costa, PhD program
Supervisor: Noël Raynal, CHU Centre de recherche Sainte-Justine

Project title: Targeting histone acetylation and oncogenes in pediatric leukemia

Description: In leukemia, epigenetic modifications are strongly altered and are responsible for gene expression aberrations. My strategy is to repurpose a cardiac glycoside that exhibits epigenetic and anticancer activities for the treatment of pediatric leukemia. The aim of the project is to highlight its epigenetic and anticancer mechanisms in leukemia cells.

Erlinda Fernández Díaz, PhD program
Supervisor: Frédérick Antoine Mallette, Maisonneuve-Rosemont Hospital Research Centre

Project title: Telomere defects induced by IDH1/2 mutations associated to acute myeloid leukemia

Description: Heterozygous mutations in genes encoding isocitrate dehydrogenases 1 and 2 (IDH1/2) occur in both pediatric and young adult acute myeloid leukemia (AML) and lead to the production of the oncometabolite d-2-hydroxyglutarate (2HG). D-2-hydroxyglutarate is a known inhibitor of KDM4A, a member of the JUMONJI family of lysine demethylases. Our goal is to investigate how metabolic defects associated to IDH1/2 mutations contribute to malignant transformation, through the KDM4A-mediated regulation of telomere function.

Ema Flores-Díaz, PhD program
Supervisor: Trang Hoang, IRIC

Project title: Targeting key vulnerabilities of pre-Leukemic Stem Cells in T-cell Acute Lymphoblastic Leukemia

Description: Current T-ALL treatment reduces the tumor burden; yet, it is associated with long-term deleterious side effects. My project aims to characterize the molecular and functional effects of two exciting drug candidates that have proven capable of abrogating pre-LSC survival and of overcoming the differentiation blockade imposed by oncogenes.

Ryan Killoran, Post PhD program
Supervisor: Matthew Smith Laboratory, IRIC University of Montreal

Project title: Characterization of Dimer-Activated MLL in Acute Leukemia

Description: This project aims to gain a better understanding of acute leukemia initiation and progression driven by chromosomal translocations in the mixed lineage leukemia (MLL) gene. Using a structural biology approach, the molecular mechanisms that govern dimer-induced MLL activation in leukemogenic fusions will be explored and characterized.

Shirin Lak, PhD program
Supervisor: Jean-Sébastien Delisle, Université de Montréal, Centre de Recherche HMR

Project title: Enhancing antigen specific T cell function through co-signaling modulation

Description: Allogeneic hematopoietic stem cell transplant (HSCT) is an established treatment for high risk or refractory hematological cancer patients. In this study we focus on manipulating the graft in laboratory and obtain Leukemia specific T cells capable to selectively target malignant cells more effectively through the modulation of the signals they receive during their activation.

Mathieu Neault, Post PhD program
Supervisors: Frédérick Antoine Mallette, Heather Melichar, Centre de Recherche HMR

Project title: A Genome-Wide CRISPR/cas9 Screen for the Identification of Novel Therapeutic Targets in Acute Megakaryoblastic Leukemia

Description: Paediatric acute megakaryoblastic leukemia (AMKL) is an aggressive disease associated with poor outcome. Our approach consists in the development of an AMKL mouse model. This model will serve as a tool to identify and characterize novel therapeutic targets using a whole-genome CRISPR/Cas9 knock-out screen.

Linnéa Olofsson, Post PhD program
Supervisor: Michael Tyers, IRIC

Project title: Understanding and exploiting Fbw7 genetic insufficiency in leukemia and lymphoma

Description: The central role of Fbw7 in leukemia and lymphoma suggests that Fbw7 mutations perturb the balance of pro- and anti-tumorigenic functions in the cancer cell. This project aims to analyze Fbw7-substrate interactions in living cells to understand the genetic interactions and biochemical effects of recurrent Fbw7 mutations.

Guillaume Richard-Carpentier, Clinician program
Supervisors : Josée Hébert, Hôpital Maisonneuve-Rosemont et Guy Sauvageau, IRIC

Project title: Identification and Validation of New Prognostic Markers to Improve Risk Stratification in Intermediate Risk Acute Myeloid Leukemia

Description: This project aims to refine the risk stratification of relapse in patients with intermediate risk acute myeloid leukemia (AML). We will identify new prognostic markers from the genic expression profiles of 452 AML specimens collected by the Banque de Cellules Leucémiques du Québec. Molecular tests will be developed and clinical validation will be performed in AML patients' cohorts.

Laura Simon, PhD program
Supervisor: Guy Sauvageau, IRIC

Project title: Chemogenomics of RUNX1-mutated Acute Myeloid Leukemia

Description: We recently showed that Glucocorticoids can selectively inhibit the proliferation of RUNX1-mutated Acute Myeloid Leukemia (AML) cells in vitro, a subgroup of AML associated with poor outcome. This project aims to dissect the mechanism of action of Glucocorticoids in AML and determine how RUNX1 function influences this response.

Damehan Tchelougou, PhD program
Supervisor: El Bachir Affar, Centre de Recherche de l'Hôpital Maisonneuve-Rosemont

Project title: ASXL family of transcription co-factors in Childhood leukemia

Description: ASXL 1 and ASXL2 are genes that regulate genome expression. Abnormalities affecting these genes lead to cells anarchic proliferation. The aim of our project is to identify the mechanisms by which ASXL1 and ASXL2 protect against the development of leukemia in children.

Elisa Tomellini, Post PhD program
Supervisor: Guy Sauvageau, IRIC

Project title: Expansion of human hematopoietic stem cells: improving and understanding self-renewal from the bench to the optimal cord graft

Description: Hematopoietic stem cell (HSC) transplantation is used as a therapeutic strategy for numerous hematological diseases. Human cord blood (CB) is one of the most accessible sources of HSCs but suffers from low cell number. We will exploit chemogenomic approaches to identify new HSC markers and achieve a better ex vivo expansion of HSC and progenitor cells from a single well-matched CB unit.

McGill University

Alexandre Benoit, PhD program
Supervisor: Koren Mann, Lady Davis Institute - Jewish General Hospital

Project title: The Role of mutated Ras-associating proteins and STAT6 in R-CHOP resistant DLBCL

Description: Roughly 1/3 of DLBCL patients do not respond or relapse after the standard treatment regimen, known as R-CHOP. We have identified mutations in the STAT6 and RASGRP4 proteins that were enriched in relapse/refractory DLBCL patient samples. We propose experiments to determine if these mutations lead to R-CHOP resistance, and importantly dictate response to novel targeted therapies.

Meaghan Boileau, PhD program
Supervisor: Kolja Eppert, Research Institute of the McGill University Health Centre

Project title: Identification of bioactive molecules that target acute myeloid leukemia stem cells

Description: Acute myeloid leukemia is an aggressive form of blood cancer that is sustained by chemo-resistant leukemic stem cells (LSCs). We have previously identified a LSC-specific gene expression signature. This project will use this LSC-specific signature to identify drugs that specifically target LSCs without harming normal hematopoietic stem cells.

Martin Karam, PhD program
Supervisor: Alexandre Orthwein, The Lady Davis Institute

Project title: Deciphering the role of the zing finger protein POGZ during hematopoiesis and pediatric Acute Myeloid Leukemia

Description: Fanconi Anemia (FA) patients typically develop acute myeloid leukemia (AML) at early age and succumb to bone marrow failure. REV7 is a recently identified FA predisposing factor, but little is known about its role in disease progression. I aim to decipher novel interactors of REV7 and their role in AML progression linked to FA.

Mingyi Vincent Luo, PhD program
Supervisor: Alexandre Orthwein, The Lady Davis Institute

Project title: Deciphering the role of deubiquitinases in B-cells and Diffuse Large B-cell lymphoma

Description: My project aims at determining the importance of a specific class of enzymes, called deubiquitinases (DUBs), in B-cell development and during lymphomagenesis. In particular, we have identified three distinct subgroups of DUBs based on their expression pattern in B-cells and in the most common B-cell lymphoma, diffuse Large B-cell lymphoma (DLBCL). It is our hope that these DUBs may become bio-markers to stratify different grades of pediatric DLBCL and could provide novel therapeutic targets in the treatment of B-cell lymphomas.

Abba Malina, Post PhD program
Supervisor: Alexandre Orthwein, The Lady Davis Institute

Project title: Determining clinically relevant genetic mutations that elicit resistance to chemotherapy in Burkitts lymphoma using CRISPR/Cas9

Description: Burkitt Lymphoma is a rare and aggressive cancer that commonly affects children and young adults. It is usually treated with intensive rounds of chemotherapy. While mostly effective, there are a few patients whose cancer fails to respond. We aim to uncover the genetics that distinguishes these patients using CRISPR/Cas9 genome editing technology.

Jutta Steinberger, Post PhD program
Supervisor: Jerry Pelletier, Department of Biochemistry, McGill University

Project title: Targeting MYC expression in lymphoma and leukemia

Description: A novel, phenotype-based screen engineered using CRISPR/Cas9 identified cardiac glycosides (CGs) as capable of blocking MYC oncogene expression. This project aims to identify the underlying mechanisms of action of CGs in lymphomas and leukemias and extends the ability by which to drug MYC.

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

Presenters / Liste des exposants

Rachid Abaji
Léo Aubert
Yahya Benslimane
Aubrée Boulet-Craig
Karine Bourdages
Willow Marie Burns
Marina Bury
Sophie Cardin
Jun Chen
Margaret R. Davis
Marion Dubuissez
Heather Duncan
Fida Khater
Céline M. Laumont
Vincent-Philippe Lavallée
Charles-Etienne Lebert-Ghali
Ludivine Litzler
Deanna MacNeil
Sophia Morel
Nandita Noronha
Adam-Nicolas Pelletier
Pierre Priam
Christina Sawchyn
Camille Simon
Aditi Sood
Elisa Tomellini
Claudia Wever
Kezhi Yan

Poster List / Liste des projets exposés

No.	Name	Title
PO1	**Abaji, Rachid** PhD (2016-2018)	Whole-exome sequencing identified genetic risk factors for asparaginase-related complications in childhood ALL patients
PO2	**Aubert, Léo** Post PhD (2016-2018)	Characterization of the surfaceome of hematopoietic and leukemic stem cells expanded with UM171 to improve the development of novel therapies for leukemia
PO3	**Benslimane, Yahya** PhD (2016-2018)	Investigating synthetic sensitivity to telomerase inhibition in acute lymphoblastic leukemia
PO4	**Boulet-Craig, Aubrée** PhD (2016-2018)	Cognitive functioning and mathematical abilities in long term survivors of acute lymphoblastic leukemia

PO5	**Bourdages, Karine** PhD (2015-2017)	Genome-wide CRISPR screens for Fbw7-specific genetic susceptibilities in cancer
PO6	**Burns, Willow Marie** PhD (2016-2018)	The perceived impact of pediatric cancer on parental couples' psychological status and relationship satisfaction during the survivorship period
PO7	**Bury, Marina** Post PhD (2015-2017)	The NFE2L3 (NRF3) transcription factor is regulated via the NF-kB pathway and plays a role in the early hematopoiesis
PO8	**Cardin, Sophie** Post PhD (2015-2017)	Generating human models of pediatric acute megakaryoblatic leukemia induced by the NUP98-KDM5A fusion oncogene
PO9	**Chen, Jun** Post PhD (2015-2017)	SLAMF7 is critical for phagocytosis of haematopoietic tumour cells via Mac-1 integrin
PO10	**Davis, Margaret R.** Post PhD (2016-2018)	Nuclear eIF4E stimulates 3'-end cleavage of target RNAs through a non-canonical pathway
PO11	**Dubuissez, Marion** Post PhD (2016-2018)	Role of Ikaros transcription factor in the transcription termination
PO12	**Duncan, Heather** PhD (2016-2018)	G Protein-Coupled Receptor 56 as a Potential Functional Regulator of Normal and Leukemic Human Stem Cells
PO13	**Khater, Fida** Post PhD (2016-2018)	Identification of actionable targets for refractory/relapsed childhood cancer leading to personalised targeted therapy (TRICEPS Study)
PO14	**Laumont, Céline M.** PhD (2016-2018)	Identification of potent targets for leukemia immunotherapy using a novel proteogenomic approach
PO15	**Lavallée, Vincent-Philippe** Clinician (2016-2018)	Podoplanin expression defines acute promyelocytic leukemia and contributes to fatal bleeding
PO16	**Lebert-Ghali, Charles-Etienne** Post PhD (2016-2018)	Modulation of T cell function by CD271

PO17	**Litzler, Ludivine** PhD (2015-2017)	PRMT5 is required for B cell proliferation and germinal center maintenance
PO18	**MacNeil, Deanna** PhD (2016-2018)	X-Linked Dyskeratosis Congenita Mutations affect the Dyskerin-Telomerase RNA Interaction
PO19	**Morel, Sophia** PhD (2016-2018)	Lipid and lipoprotein abnormalities in acute lymphoblastic leukemia survivors
PO20	**Noronha, Nandita** PhD (2016-2018)	Identification of the molecular pathways mediating the anti-AML activity of statins
PO21	**Pelletier, Adam-Nicolas** Post PhD (2015-2017)	High-throughput characterization of transcription factor variants in the context of Acute Myeloid Leukemia
PO22	**Priam, Pierre** PhD (2016-2018)	Loss-of-function Mutations in SMARCD2 in Patients Suffering from Specific Granule Deficiency
PO23	**Sawchyn, Christina** PhD (2016-2018)	Exploring the role of the KDM4A Jumonji-C demethylase at transcriptional enhancers in pediatric acute myeloid leukemia
PO24	**Simon, Camille** PhD (2015-2017)	Ezh1 and Ezh2 are essential regulators of normal and leukemic hematopoietic stem cells
PO25	**Sood, Aditi** Post PhD (2016-2018)	Optimizing the T cell repertoire for adoptive immunotherapy
PO26	**Tomellini, Elisa** Post PhD (2015-2017)	Phosphoproteomic and genetic analysis of UM171-treated hematopoietic stem and progenitor cells
PO27	**Wever, Claudia** Post PhD (2016-2018)	BH3 Profiling of Primary Non-Hodgkin Lymphoma Samples
PO28	**Yan, Kezhi** PhD (2016-2018)	Role of the multidomain epigenetic regulator BRPF1 in leukemia development

PO1

Title: Whole-exome sequencing identified genetic risk factors for asparaginase-related complications in childhood ALL patients

Author: Rachid, Abaji

Affiliation: Vincent Gagné, Chang Jiang Xu, Jean-François Spinella, Francesco Ceppi, Caroline Laverdière, Jean-Marie Leclerc, Daniel Sinnett, and Maja Krajinovic; Department of Pharmacology at the University of Montreal and Research Center of CHU Sainte-Justine.

Key words: Acute lymphoblastic leukemia; asparaginase; exome-wide association; pharmacogenetics; whole-exome sequencing.

Background information: Allergy, pancreatitis and thrombosis are common side-effects of childhood acute lymphoblastic leukemia (ALL) treatment that are associated with the use of asparaginase (ASNase), a key component in most ALL treatment protocols.

Purpose of the study: Our aim is to identify of genetic markers underlying the inter-individual variability in the susceptibility to ASNase-induced complications in the treatment of childhood ALL.

Methods: Starting with predicted functional germline variants obtained through whole-exome sequencing (WES) data of the Quebec childhood ALL cohort (N=302) we performed exome-wide association studies with ASNase-related toxicities. A subset of top-ranking variants was further confirmed by genotyping followed by validation in an independent replication group (N=282); except for thrombosis which was not available for that dataset.

Results: SNPs in 12 genes were associated with ASNase complications in discovery cohort including 3 that were associated with allergy, 3 with pancreatitis and 6 with thrombosis. The risk was further increased through combined SNP effect ($p \leq 0.002$), suggesting synergistic interactions between the SNPs identified in each of the studied toxicities. Interestingly, rs3809849 in the MYBBP1A gene was associated with allergy ($p = 0.0006$), pancreatitis ($p=0.002$), thrombosis ($p=0.02$), event-free survival ($p=0.02$) and overall survival ($p=0.003$). Furthermore, rs11556218 in IL16 and rs34708521 in SPEF2 were both associated with thrombosis ($p=0.01$ and $p=0.03$, respectively) and pancreatitis ($p=0.02$). The association of SNPs in MYBBP1A, IL16 and SPEF2 genes with pancreatitis as well as combined SNPs effect was replicated in the validation cohort ($p \leq 0.05$).

Conclusions: The present work demonstrates that using WES data is a successful "hypothesis-free" strategy for identifying significant genetic markers modulating the effect of the treatment in childhood ALL. It also highlights the importance of the synergistic effect of combining risk loci which had the highest power to predict the development of pancreatitis.

PO2

Title: Characterization of the surfaceome of hematopoietic and leukemic stem cells expanded with UM171 to improve the development of novel therapies for leukemia

Author: Léo Aubert, Post PhD 2016-2018

Affiliation: Dr. Philippe P. Roux, Department of Pathology and Cell Biology, Institute for Research in Immunology and Cancer (IRIC) – Université de Montréal

Keywords: Leukemia, Hematopoietic Stem Cells (HSCs), Surfaceome, UM171, Therapeutic targets

Background Information: Hematopoietic stem cell transplantation (HSCT) has become a potent life-saving procedure for both adults and children with various hematological malignancies, including acute myeloid leukemia (AML). Umbilical cord blood (CB) appears as one of the most attractive sources of HSCs for transplantation, due to its immature immune system and widespread availability. Unfortunately, many patients are deprived from this therapeutic strategy as the low stem cell dose in CB units results in delayed engraftment and compromises transplantation outcome. Our collaborators from Guy Sauvageau's group, have recently discovered a small-molecule, UM171, which stimulates the expansion of CB HSCs ex vivo. Interestingly, UM171 can also promote the in vitro maintenance of leukemic stem cells (LSCs), which are considered one of the major causes of therapeutic failure in AML. However, the unavailability of specific surface markers that can prospectively identify HSCs and LSCs is still a major hurdle for the optimization of CB grafts and the development of new targeted therapies.

Purpose of the study: The aim of this collaborative research project is to identify new HSC and LSC surface markers using quantitative proteomic methods. These results will help better purify and characterize HSCs and LSCs in vitro, with the final purpose of helping patients with leukemic disorders either through the optimization of HSCT or the uncovering of new therapeutic targets.

Methods: To discover novel and reliable HSC and LSC surface markers, we have optimized and adapted a cutting-edge chemoproteomic approach based on the labeling of cell surface proteins with cell-impermeable biotin reagents, their subsequent purification with avidin chromatography, and quantification using label-free quantitative proteomics with liquid chromatography-tandem mass spectrometry.

Results: As a proof of concept experiment, we used this quantitative proteomic approach in the hematopoietic cell line OCI-AML5 in combination with UM171-induced cell expansion. We found that UM171 promotes the upregulation of more than 35 cell surface proteins. Among them, we identified the endothelial protein C receptor (EPCR) which we recently characterized as a novel surface marker for the HSC population derived from CB expanded with UM171. To optimize this method for the analysis of CB cells, we performed a OCI-AML5 cell titration and determined the minimal number of hematopoietic cells required. We then performed a

surface proteomics analysis of cell populations from UM171-expanded CB units and sorted according to levels of CD34 and EPCR. Preliminary results reveal the enrichment in more than 100 surface proteins in the CD34+EPCR+ population, and several of these proteins are currently being tested as potential new markers for HSCs.

Conclusion: Altogether, these results validate the surfaceome approach for the identification of novel HSC- and LSC-specific surface molecules using CB units and primary AML specimens, respectively.

PO3

Title: Investigating synthetic sensitivity to telomerase inhibition in acute lymphoblastic leukemia

Author: Yahya Benslimane

Affiliation: Thierry Bertomeu, Jasmin Coulombe-Huntington, Corinne Saint-Denis, Mike Tyers, Lea Harrington. Department of Medicine, University of Montreal, Institute for Research in Immunology and Cancer (IRIC)

Keywords: Acute lymphoblastic leukemia, Telomeres, Telomerase inhibition, CRISPR, genome-wide screening

Background information: Chromosome ends (telomeres) shorten with every cellular division in normal somatic cells while leukemic cells re-activate telomerase (TERT) to counteract this shortening and become immortal. B-cell acute lymphoblastic leukemia (B-ALL) is a form of ALL characterized by high telomerase activity and short average telomere length, suggesting that B-ALL would be more susceptible to telomerase inactivation. However, strategies targeting telomerase suffer from a 'therapeutic lag' due to the need for cells to proliferate before telomeres become critically short.

Purpose of the study: We hypothesize that telomerase inhibition can be combined with the disruption of other gene functions to increase the damaging potential of eroded telomeres. This would in turn trigger cell death in ALL cells at a higher rate than in normal blood cells.

Methods: A potent telomerase inhibitor (BIBR1532) is used to induce telomere shortening in NALM-6 cells (a pre-B ALL cell line). These cells were transduced with a lentiviral whole-genome targeting sgRNA library to generate a pooled collection of individual gene disruptions across the entire genome. Upon CRISPR-mediated knockout and next-generation sequencing, it is possible to assess the entire cell population's fitness in response to telomerase inhibition.

Results: A pilot chemo-genomic screen was performed in the presence of BIBR1532 using conditions previously reported in the literature. This pilot screen uncovered genes that showed a BIBR1532-dependant difference in fitness upon a treatment of 20 days. The effect of BIBR1532 on NALM-6 cell proliferation and telomere length were measured as well. The information from this characterization will guide the choice of the optimal parameters to use for a secondary whole-genome screen. Finally, a CRISPR knockout of TERT was performed in NALM-6 cells in order to study the effect of complete ablation of telomerase activity on cell proliferation.

Conclusion: Gene knockouts that are specifically depleted in the presence of BIBR1532 will be further validated using individual sgRNAs to confirm synthetic sensitivity to telomerase inhibition in NALM-6 cells as well as other ALL cell lines. This will be used to design novel alternative therapies that directly target telomerase in B-ALL. These combinatorial strategies are of special interest to acute lymphoblastic leukemia patients who relapsed due to resistance to the currently available therapies.

PO4

Title: Cognitive functioning and mathematical abilities in long term survivors of acute lymphoblastic leukemia

Author: Aubrée Boulet-Craig and Julie Laniel

Affiliation: Sarah Lippé (supervisor), Département de psychologie, Université de Montréal

Keywords: mathematics, visual short-term memory, magnetoencephalography, acute lymphoblastic leukemia

Background information: Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children. Because of major improvements in treatment protocols over the last decades, almost 90% of children diagnosed with ALL now survive at least 5 years post-diagnosis. However, ALL treatments can cause long-term cognitive difficulties, like lowered IQ, working memory and mathematics performance (Iyer et al., 2015). Some risk factors like age at diagnosis can increase the probability of developing deficits.

Working memory seem to predict deficits in mathematics in the healthy population. Moreover, visual-short term memory (VSTM) brain activity is linked to performance in mathematics in healthy adults (Boulet-Craig et al., 2017). It has also been demonstrated that visual memory was related to mathematics in ALL survivors (Kaemingk et al., 2004).

Purpose of the study: Main objectives were to investigate the link between age at diagnosis and cognitive functioning years later and the relation between VSTM neural correlates and performance in mathematics in a cohort of ALL survivors.

Methods: A total of 48 ALL survivors and 25 controls completed a neuropsychological evaluation and a VSTM task in magnetoencephalography. To investigate neural correlates of VSTM, we computed the contralateral activation (CA), which reflects retention of information in short-term memory. It appears during the retention period of a VSTM task and is maximal on parieto-occipital sensors (Boulet-Craig et al., 2017).

Results: IQ ($p=0.001$, $R^2=0.25$), mathematical reasoning ($p=0.004$, $R^2=0.16$) and calculation fluency ($p=0.004$, $R^2=0.17$) were predicted by age at diagnosis. A younger age at diagnosis lead to lower results on our neuropsychological measures. Mathematical reasoning ($p=0.041$, $R^2=0.16$) and calculation fluency ($p=0.004$, $R^2=0.29$) were predicted by CA in controls. However, there were no relation between performance in mathematics and CA in ALL survivors. Preliminary analyses at the source level hence show that ALL survivors have reduced parieto-occipital activity but enhanced frontal activity compared to controls during the retention phase of our VSTM task.

Conclusion: Our results show that age at diagnosis significantly predicts mathematical skills years after remission. Moreover, our results show that the relation between VSTM neural correlates and performance in mathematics found in healthy adults does not exist in ALL survivors. ALL survivors may use different strategies to perform in VSTM tasks, which are not related to mathematical skills.

PO5

(absent)

Title: Genome-wide CRISPR screens for Fbw7-specific genetic susceptibilities in cancer

Authors: Karine Bourdages[1], Thierry Bertomeu[1], Jasmin Coulombe-Huntingdon[1] & Mike Tyers[1]

Affiliation: [1]Institute for Research in Immunology and Cancer, Department of Medicine, Université de Montréal, Montreal, Canada

Keywords: Acute lymphoblastic leukemia, Fbw7, CRISPR, genome-wide, synthetic lethal

The tumor suppressor Fbw7 is one of the most frequently mutated genes in cancer. For example, Fbw7 is mutated in 31% of T-cell acute lymphoblastic leukemia. Fbw7 is a critical substrate recognition subunit of the SCF ubiquitin ligase complex, and targets dozens of substrates for degradation, including the oncogenes Cyclin E, MYC, JUN and NotchICD. Loss of Fbw7 function can thus lead to increased proliferation, stemness and/or genome instability. An Arg-rich pocket on the WD40 domain of Fbw7 binds to phosphorylated degron motifs on substrates. Cancer-associated mutations often alter these Arg residues (termed Fbw7ARG) and appear to act in a partially dominant negative manner over the remaining wild type copy. We hypothesize that cancer cells mutated for Fbw7 may be vulnerable to loss of other gene functions, termed synthetic lethality.

We carried out systematic Fbw7+/- synthetic lethal screens in T-ALL and other blood cancer cells lines in order to uncover synthetic lethal genetic interactions with Fbw7. We constructed a whole-genome CRISPR pooled library of 278,000 sgRNAs that covers 22,959 known or putative human genes, as well as all documented protein splice isoforms. Baseline screens in a human pre-B ALL cell line that is wild type for Fbw7 surprisingly revealed that Fbw7 was essential in this particular cell line. In order to systematically identify Fbw7-specific genetic interactions that may be selected for during cancer progression, we have performed positive selection screens to rescue fitness defects associated with the loss of Fbw7. Conversely, to identify potential genetic susceptibilities of Fbw7+/- or Fbw7ARG/+ cancer cells, we have performed depletion screens against different leukemic cell lines. Collectively, these genome-wide screens will define the genetic interaction network for Fbw7 and identify candidate novel targets for the design of small molecule inhibitors that are selective for cancers with Fbw7 mutations.

PO6

Title: The perceived impact of pediatric cancer on parental couples' psychological status and relationship satisfaction during the survivorship period

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Keywords: pediatric cancer, couples, distress, relationship changes, interdependence

Background information: Follow-up studies suggest that the psychosocial impact of pediatric cancer on parents often extends beyond the end of their child's cancer treatments, and parents can continue to experience both individual and dyadic (couple-based) effects.

Purpose of the study: In a long-term study of parents of children with acute lymphoblastic leukemia (ALL), we aimed to: 1) describe couples' adjustment (psychological distress, relationship adjustment), 2) describe the perceived impact of cancer on couples' relationships, and 3) identify to what extent the perceived impact of cancer on the couple is related to both parents' long-term adjustment.

Methods: Participants in this study were childhood ALL survivors and their parents, who were in 'intact' couples (i.e. together during the illness and at recall) (n =100). Survivors' medical information was collected from medical records and parents completed questionnaires (Brief Symptom Inventory-18, abbreviated Dyadic Adjustment Scale, and Impact of Cancer on the Couple). Mothers and fathers' scores were compared (e.g., repeated-measures MANOVAs). Using the Actor-Partner Interdependence Model (APIM), we examined the degree to which a parent's perceived changes in relationship dynamics were associated with their own adjustment (actor effects), and their partner's adjustment (partner effects).

Results: Distress was normative in this sample of parents with frequencies ranging from 2 to 21%. Generally, mothers and fathers agreed on their reported relationship satisfaction, and the perceived nature of relationship changes (positive, negative, or no change) following the illness. Findings from dyadic models indicate that while mothers' adjustment was self-related (relating to their perceived relationship changes), fathers' adjustment was primarily other-related (relating to their partner's perceptions).

Conclusion: Given our findings, it appears that mothers may act as a bridge connecting the illness experiences of survivors and fathers. This could explain why mothers' perceptions of relationship changes influence their partners' long-term adjustment, but the reverse is not true for fathers.

Acknowledgments: Cole Foundation (Montreal), CIHR, FRQs

PO7

Title: The NFE2L3 (NRF3) transcription factor is regulated via the NF-kB pathway and plays a role in the early hematopoiesis

Author: Marina Bury

Affiliation: Joo Yeoun Park, Yusra K. Al Mosuli, Anna Derjuga, Dr. Volker Blank, Faculty of Medicine, McGill University, Lady Davis Institute for Medical Research

Background information: Nuclear factor, erythroid 2-like 3 (NFE2L3 or NRF3) is a basic leucine zipper transcription factor that belongs to the cap'n'collar family. A series of data link NFE2L3 to hematopoietic cell function and malignancies. Our previous study showed that, following benzo[a]pyrene exposure, Nfe2l3-/- mice exhibited increased mortality and morbidity and developed a higher number of lymphomas. NFE2L3 transcripts have also been shown to be highly expressed in human lymphoma specimens.

Keywords: transcription factor, hematopoiesis, lymphoma

Purpose of the study: We propose to elucidate the molecular mechanisms governing the regulation of NFE2L3 in hematopoietic cells and its role in hematopoiesis.

Methods: To investigate the regulatory pathways of NFE2L3, Jurkat T-lymphocyte and Namalwa Burkitt lymphoma cells were treated with known modulators of hematopoietic cell function, including tumor necrosis factor-alpha (TNFa). To gain insights into the role of NFE2L3 in hematopoiesis, we analyzed Nfe2l3-/- mice by flow cytometry and/or complete blood count (CBC) measurements.

Results: TNFa increased the protein expression level of NFE2L3 as well as the phosphorylation of IkBa in both Jurkat and Namalwa cells. BAY 11-7082, an inhibitor of IkBa phosphorylation and JSH23, an inhibtor of the transcriptional activity of NF-kB, respectively decreased the induction of NFE2L3 levels by TNFa. Moreover, we found that NFE2L3 expression was highly elevated at both transcript and protein levels in a subset of activated B-cell-like (ABC) and germinal center B-cell-like (GCB) subtypes of diffuse large B-cell lymphoma (DLBCL) cell lines. In our vivo experiments, Nfe2l3-/- mice exhibited significant differences in HSC and progenitor cell pools in the bone marrow, but not in mature blood cells, when compared to wild-type animals.

Conclusions: We showed that TNFa is a positive regulator of NFE2L3 expression in hematopoietic cells. This induction is likely mediated by the NF-kB pathway. Since NF-kB signaling is crucial to the pathogenesis of many cancer types, we expect NFE2L3 to contribute to the malignancy. We found increased expression of NFE2L3 in a subset of ABC and GCB subtype DLBCL cell lines. In addition, NFE2L3 most likely plays a role in the early stages of hematopoiesis.

PO8

Title: Generating human models of pediatric acute megakaryoblatic leukemia induced by the NUP98-KDM5A fusion oncogene.

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Keywords: Megakaryoblastic, pediatric, model leukemia, NUP98-KDM5A

Background: Pediatric acute megakaryoblastic leukemia (AMKL) accounts for 10% of childhood acute myeloid leukemia (AML) cases and remains a high fatality cancer. CBFA2T3-GLIS2, NUP98-KDM5A, RBM15-MKL1 and MLL gene rearrangements are recurrent aberrations that are mutually exclusive and found at similar frequencies in half the cases of pediatric AMKL. Stagnating cure rates (<50%) for AMKL are in part attributed to the paucity of patient samples, and the lack of relevant models to gain insight into the disease, especially in the physiologically relevant context of human cells. To investigate NUP98-KDM5A driven leukemogenesis, human cell lines and mouse models were engineered using overexpression of the chimeric oncogene in CD34+ cord blood (CB) stem/progenitor cells.

Purpose of the study: To generate human models of high fatality AMKL to identify biomarkers and putative drug targets.

Methods: The cDNA of the NUP98-KDM5A (NKDM5A) fusion, consisting of the 5' portion of the nuclear pore protein nucleoporin 98 (NUP98) gene fused to the 3' portion of the histone lysine demethylase 5A (KDM5A) encoding gene, was cloned into a lentiviral vector carrying a GFP reporter gene. Freshly isolated CB-CD34+ (day 0) cells were transduced with either NKDM5A or control (CTL) vectors. Xenotransplantation of 75% of day 7 cells in immunodeficient mice was performed to generate the in vivo models.

Results: Overexpression of NKDM5A led to maturation block and increased cell proliferation in vitro compared to CTL, as assessed by cytological examination of cells, serial tracking of increasing CD34+GFP+ cell fractions and lack of a c-KIThi differentiated mast cell population. An overriding NKDM5A CD34+GFP+ immature cell population was present by day 42 of culture (up to 94% of cells), while CD34+GFP+ cells were significantly decreased in the CTL conditions (<10% where mast cells predominated. Clonogenic activity was markedly increased in NKDM5A cells (colony forming cell frequency of 1:3 vs 1:200 in CTL, at day 88 of culture). Transcriptomic profiling of selected cell lines closely matched that of NKDM5A bearing pediatric AMKL samples. Notably, there was an up-regulation of HOXA and HOXB gene clusters, of other transcription factors, epigenetic regulators, cell surface receptors and kinases. In vivo, recipient mice developed AMKL by 32-40 weeks post transplantation, with marrow cavity infiltrated by hCD45loCD61+GFP+ leukemic blasts, with typical megakaryoblastic morphology (n=2 expts). The leukemic blasts were also detected in blood (5%), and in enlarged spleen (0.2%). Secondary transplanted AMKL cells were detected in bone marrow of recipient mice 41 weeks post-transplantation.

Conclusion: Overexpression of the NKDM5A chimeric oncogene in cord blood CD34+ cells can thus be used to engineer human cell lines and xenograft models to investigate a poor prognostic subgroup of pediatric AMKL. By crossing expression profiles of patient samples, cell lines and xenograft models, a list of potential NKDM5A direct targets was elaborated. The engineered cell lines pave the way to genetic and chemical screens, to identify leukemia specific functional dependencies and drug targets.

PO9

Title: SLAMF7 is critical for phagocytosis of haematopoietic tumour cells via Mac-1 integrin

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Keywords: checkpoint blockade, immunotherapy, lymphoma, leukemia, phagocytosis, macrophages, CD47, SIRPα, SLAMF7, Mac-1 integrin

Background information: Cancer cells elude antitumor immunity through multiple mechanisms, including up-regulated expression of ligands for inhibitory immune checkpoint receptors. Phagocytosis by macrophages plays a critical role in cancer control. Therapeutic blockade of SIRPα, an inhibitory receptor on macrophages, or of its ligand CD47 expressed on tumour cells, improves tumour cell elimination in vitro and in vivo, suggesting that blockade of the SIRPα-CD47 checkpoint could be useful to treat human cancer. However, the pro-phagocytic receptor(s) responsible for tumour cell phagocytosis is (are) largely elusive

Purpose of the study: We were intended to discover the receptor(s) responsible for recognize and clear tumour cells on macrophage and further explore the related mechanisms, which will help to develop novel strategy to treat human cancer by immunotherapy.

Methods: We combined genetic approaches, cell biology, immune signalling and biochemistry to screen the phagocytic receptor and study the mechanism of phagocytosis by macrophages.

Results: We found that macrophages were much more efficient at phagocytosis of hematopoietic tumour cells, compared to non-hematopoietic tumour cells, in response to SIRPα-CD47 blockade. Using a mouse lacking the SLAM family of homotypic hematopoietic cell-specific receptors, we determined that phagocytosis of hematopoietic tumour cells during SIRPα-CD47 blockade was strictly dependent on SLAM family receptors in vitro and in vivo. In both mouse and human cells, this function required a single SLAM family member, SLAMF7 (also named CRACC, CS1, CD319), expressed on macrophages and tumour cell targets. In contrast to most SLAM receptor functions, SLAMF7-mediated phagocytosis was independent of SAP adaptors. Instead, it depended on the ability of SLAMF7 to interact with integrin Mac-1 and utilize signals involving immunoreceptor tyrosine-based activation motifs (ITAMs).

Conclusion: These findings elucidate the mechanism by which macrophages engulf and destroy hematopoietic tumour cells. They also reveal a novel SAP adaptor-independent function for a SLAM receptor. Lastly, they suggest that patients with tumours expressing SLAMF7 are more likely to respond to SIRPα-CD47 blockade therapy.

PO10

Title: Nuclear eIF4E stimulates 3'-end cleavage of target RNAs through a non-canonical pathway

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Keywords: eIF4E, 3' processing, cleavage, polyadenylation, oncogenesis

Background: Eukaryotic translation initiation factor 4E, eIF4E, is an oncogene that is elevated in 30% of malignancies, as well as highly upregulated in M4/M5 acute myeloid leukemia (AML) and a subset of lymphoma. eIF4E activity is known in translation, stability of mRNA in P-bodies and nuclear mRNA export of a subset of m7G- capped and spliced RNAs that contain a unique regulatory sequence in the 3' untranslated region (3'UTR). Interestingly, initial studies in our laboratory suggest an additional role for eIF4E and co-factors in 3' processing through cleavage events associated with alternate polyadenylation (APA). APA is the use of alternative cleavage sites, increased cleavage activity and/or alterations in polyA tail length. Importantly, APA plays a major role in the complexity and decoupling of the transcriptome and the proteome producing varying isoforms in the transcript and/or the 3' UTR. This profoundly impacts on the proteome and in turn, contributes to oncogenesis.

Purpose of the Study: The aim of this study is to investigate the role of eIF4E in 3' formation/processing using fundamental molecular biology techniques and determine the biochemical mechanism through which eIF4E employs this pathway.

Methods: Stable cell lines were generated with model RNA constructs containing the unique regulatory sequence in the 3'UTR region, in both vector controls and eIF4E overexpressing cells. Cells were fractionated into nuclear and cytoplasmic components. Cleavage assays were performed via RTqPCR and primers were designed to flank the 5' and 3' end of the regulatory region, in the nuclear fraction. We further performed immunoprecipitation (IP) assays, RNAi and anti-sense morhpolino (AMO) assays to identify key co-factors in this process.

Results: Our studies show that eIF4E drives cleavage in select mRNA targets with the unique regulatory 3'UTR sequence and subsequently increased protein expression of the targets. Importantly, the mechanism at which this pathway is activated involves eIF4E physically associating with cleavage enzyme, CPSF3, and other co-factors not normally associated with 3' processing such as UsnRNAs and PRP splicesomal subunits. In addition, though RNA-IP and PCR-directed mutagenesis, we were able to further define the fundamental sequence requirements of the eIF4E targets to stimulate the pathway.

Conclusions: These are the first studies to show that eIF4E alters cleavage of target transcripts, therefore demonstrating an active role of eIF4E in 3'processing. This positions eIF4E upstream of mRNA export. Further we dissected the pathway eIF4E uses to increase cleavage of transcripts. whereby eIF4E interacts with key splicesomal and cleavage factors to affect APA in select transcripts. Importantly, this pathway is stimulated in the nucleus and therefore affects a select subset of transcripts and has substantial impacts on protein expression. Thus, we not only identified a novel function of eIF4E but also a novel polyadenylation pathway, which impact on a broad range of oncoproteins.

PO11

(absent)

Title: Role of Ikaros transcription factor in the transcription termination

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Keywords: Ikaros, NuRD complex, Transcription termination, Leukemia

Background information: The transcription factor and tumor suppressor Ikaros is essential in the development of lymphoid and myeloid cells. It is encoded by IKZF1 gene which is frequently mutated or deleted in various forms of leukemia, including 15% of cases of acute lymphoblastic leukemia (ALL). The mutation/deletion of Ikaros is a poor prognosis factor for pediatric ALLs. Thus, define the role of Ikaros in hematopoiesis should help to find new therapies necessary to treat children with ALL characterized by a mutation/deletion of Ikaros.

The Ikaros protein, associated with the NuRD (Nucleosome Remodeling and Deacetylase) complex acts as a transcriptional repressor or activator. However, for many of its target genes, absence of Ikaros results in a change of expression rather than full repression or expression of genes.

Purpose of the study: We showed that Ikaros-NuRD complex regulates transcription elongation by interacting with the PTEFb elongation factor. In addition, a proteomic study suggests that Ikaros will be involved in the regulation of transcription termination. Indeed, mass spectrometry analysis of proteins associated with Ikaros identifies several transcription termination factors such as XRN2, TTF2, SETX and polyadenylation factors CPSF1-5, SYMPK and PABN1 (Bottardi et al., 2014). Moreover, a recent study showed that PTEFb phosphorylates and regulates the activity of XRN2, an exoribonuclease required for stopping the RNA Polymerase II (Pol II) synthesis and for RNA cleavage at TTS (Transcription Termination Site). In summary, the Ikaros-NuRD complex is directly involved in two key stages of transcription: initiation and elongation. However, the role of Ikaros in transcription termination remains unknown.

We therefore suggest that Ikaros associating with NuRD and PTEFb during elongation would also be involved in the termination of transcription and more precisely in the activation of XRN2.

Methods: The objectives of the study are: (i) study the interaction between Ikaros-NuRD complex and factors of transcription termination; (ii) determine if Ikaros allows effective recruitment of these proteins at TTS to facilitate transcription termination; and (iii) determine if Ikaros-PTEFb complex influences XRN2 activity at Ikaros target genes.

We used the murine progenitor cells G1E2 and progenitor cells (negative lineage: Lin-) of fetal liver from embryonic mice (e14.5) Ikaros WT and Ikaros null (knockout -/-). To test interactions between Ikaros and proteins of transcription termination, we realized reciprocal co-immunoprecipitation experiments (Co-IP). We also examined the binding of Ikaros-NuRD complex with termination proteins at TTS of Ikaros target

genes by chromatin immunoprecitiation (ChIPqPCR). An analysis by ChIP-Seq will also be performed to determine the presence of Ikaros at TTS regions in the genome. Then, we will investigate whether the lack of Ikaros in the fetal liver cells Lin- Ikaros -/- influences the recruitment of NuRD complex, PTEFb and termination factors to TTS of identified target genes. Next, a gene expression analysis at genome-wide GRO-Seq (Global Run-On Sequencing; Core et al., 2008) will be realized to determine the effect of Ikaros absence on the production of primary RNA. This technique will define if selected TTS are modified in the absence of Ikaros. In summary, we will determine whether Ikaros can regulate the expression of its target genes by modulating the transcription termination. Finally, to identify the role of Ikaros-NuRD-PTEFb complex in regulating XRN2 activity, we will introduce IkD6 isoform which exon 6 is deleted in cells Ikaros -/- and determine if XRN2 activity is modulated at Ikaros target genes. This isoform interacts with NuRD complex but not with PTEFb elongation factor.

Results: Our results of Co-IP experiments in chromatin extracts of G1E2 cells suggest that Ikaros-NuRD complex can interact with XRN2 and TTF2. Then, to study the recruitment of these proteins on Ikaros target genes, we selected three genes: KIT, a known Ikaros target gene, TAL1 (T-cell acute lymphocytic leukemia protein 1) and E2F4 (E2F transcription factor 4). TAL1 and E2F4 were identified from a ChIPseq Ikaros realized in B cells (Farnham et al., 2012). The results of ChIP suggest that Ikaros-NuRD-PTEFb are in complex with TTF2 and are recruited simultaneously at the TSS. Furthermore, they are recruited with XRN2 at TTS of TAL1 and E2F4 genes. Finally, the study of expression of these two genes suggests that Ikaros influence the transcription termination of these genes. In fact, when Ikaros is absent, we detect readthrough transcripts after TTS of gene suggesting a defect of termination.

Conclusion: In contrast to initiation and elongation, the transcription termination is poorly understood. In many cancers, this step of transcription is defective with protein expression deregulation like overexpression of XRN2 and SETX, respectively, in acute myeloid leukemia (AML) and in B-cell lymphomas. This project will determine Ikaros role in transcription termination and in this case, Ikaros would be the first transcription factor involved in the three steps of transcription. Understand the transcriptional regulation mechanisms of Ikaros will define its importance in compare to its absence in pediatric B-ALLs. This study will allow important new knowledge for the development of therapies to treat hematopoietic disorders in which Ikaros is dysregulated or mutated.

PO12

Title: G Protein-Coupled Receptor 56 as a Potential Functional Regulator of Normal and Leukemic Human Stem Cells

Author: Heather Duncan, PhD 2016-2018

Affiliation: Kolja Eppert, Research Institute of the McGill University Health Centre

Keywords: Acute myeloid leukemia; G protein-coupled receptor 56; hematopoietic stem cells; leukemic stem cells; functional assays

Background information: Leukemic stem cells (LSCs) sustain acute myeloid leukemia (AML) and must be eliminated to cure a patient. Improved understanding of the molecular biology of these cells is required to develop targeted therapies. Human LSCs share gene expression profiles and molecular regulators with hematopoietic stem cells (HSCs). GPR56 has been implicated in murine HSC development and regulation. GPR56 also affects adhesion, migration and differentiation in AML cell lines. Overexpression of GPR56 has been shown to accelerate the induction of AML in mice. In human AML samples, GPR56 is a novel LSC marker associated with higher xenograft efficiency.

Purpose of the study: This study aims to establish the role of GPR56 in primary human LSCs and HSCs. Insight into the function of GPR56 as a stem cell regulator in these populations will reveal its potential as a target for therapy.

Methods: Expression of GPR56 in flow sorted samples of normal and leukemic blood was assessed by qRT-PCR. Microarray expression data from 3 cohorts, one including cytogenetically abnormal AML, was analyzed to determine the association between GPR56 expression and cytogenetic risk or outcome. Cord blood derived HSCs were transduced with GPR56 overexpression lentivirus then injected into immune-deficient mice. Engraftment was measured after 12 weeks by flow cytometry.

Results: Expression of GPR56 was higher in LSCs, normal HSCs, and normal progenitors versus mature populations in normal or leukemic blood. There was a positive correlation between GPR56 expression and poor outcome ($P<0.006$). Expression of GPR56 was higher in intermediate and high cytogenetic risk samples. Over-expression of GPR56 conferred a significant engraftment advantage versus hRluc control ($p<0.0001$). This advantage was maintained in secondary 12-week transplants ($p=0.0375$) with unaltered percentage of stem and progenitor cells indicating self-renewal of stem cells.

Conclusions: These data suggest that GPR56 may regulate human blood stem cells, including LSCs, and contribute to poor outcome in human acute myeloid leukemia through effects on leukemic stem cells. We will continue to explore a functional role for GPR56 through in vitro and in vivo assays in order to provide insight into the molecular regulation of primary human HSCs and LSCs.

PO13

Title: Identification of actionable targets for refractory/relapsed childhood cancer leading to personalised targeted therapy (TRICEPS Study)

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Affiliation: Pr. Daniel Sinnett, CHU Sainte-Justine, Hemato-Oncology

Keywords: Peadiatric cancer, Relapse, genomic-driven targeted therapy, KMT2E-ASNS, PTT

Background information: Childhood cancer is a group of heterogeneous complex diseases. Although 80% of these children are cured with conventional therapies, it remains the first cause of death among children in Western countries. A significant number of refractory/relapse patients will eventually succumb to their disease and the lack of therapeutic advances for these patients is even more worrisome. Indeed, no significant progress has been noted over the last decade for these patients, urging the need for new and more effective therapeutic approaches. Precision medicine and more effective personalized targeted therapies (PTT) are a major breakthrough leading to increased cure rates and decreased treatment-related morbidity and mortality for the patients with refractory or relapsed tumours.

Purpose of the study: To address this challenge, the TRICEPS study was initiated on April 2014 at the Sainte-Justine UHC (Montreal, Canada) with an overreaching goal to explore the feasibility of performing genomic-driven targeted therapy in pediatric and adolescent (aged 0-21 years) patients with relapsed or refractory childhood cancer.

Methods: This study offers in-depth genomic and transcriptomic investigation of patient's tumoral material to identify patient-specific alterations and actionable driver mutation(s) that can be targeted with approved targeted drug and within a reasonable clinically-relevant timeframe to assess the feasibility of going from biopsy to a detailed tumour analysis report.

Results: Over a period of 30 months, 44 relapsed/refractory cancer patients were recruited. Twenty-two of them underwent extensive genomic investigation (exomic and transcriptomic sequencing) within a median timeframe of 9.7 weeks from patient enrolment to return of results. In all 22 patients, we have identified clinically relevant genomic alterations (SNVs, indels, fusions, CNAs) and relapse-specific mutations influencing patient management and providing options for personalized interventions. We assessed the functional impact of some of these cancer-specific alterations. This was the case of a novel relapse-specific rearrangement, identified on relapsed childhood ETP-ALL, and leading to asparagine synthetase (ASNS) up-regulation through a promoter exchange. The expression of this fusion was associated with reduced apoptosis following L-asparaginase treatment (Khater et al., 2017).

Conclusion: This study shows that PPT based on next generation sequencing technology is a powerful approach that could be implemented in the clinic within a foreseeable future to guide treatment of hard-to-treat childhood cancers and to further improve patient care and outcomes.

PO14

Title: Identification of potent targets for leukemia immunotherapy using a novel proteogenomic approach

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Keywords: Antigens, CD8 T cells, immunotherapy, leukemia, MHC I molecules

Background information: MHC I peptide complexes (pMHC) are presented at the surface of all nucleated cells and play a central role in CD8 T cell immunosurveillance. The pMHC presented by medullary thymic epithelial cells (mTEChi) are essential to eliminate self-reactive CD8 T cells in a process known as central tolerance. On leukemic cells, non-tolerogenic pMHC are referred to as leukemia-specific antigens (LSAs) since they are absent from mTEChi as well as any other normal cell.

Purpose of the study: Despite their clinical relevance and because they derive from patient-specific mutations or patient-specific gene expression changes, identifying LSAs remains a challenge.

Methods: We performed RNA-sequencing on EL4 cells, a murine thymoma, along with two normal samples, namely mTEChi and thymocytes. The resulting RNA-sequencing reads were chopped into smaller sequences, called k-mers, in order to extract the EL4-specific ones. To decipher which of these EL4-specific sequences did generate LSAs, we translated them in silico and used this as a database to identify pMHC eluted from EL4 cells and sequenced by mass spectrometry.

Results: This proteogenomic analysis lead us to identify 5 LSAs truly presented by EL4 cells. While assessing their immunogenic potential, we observed that mice immunized against 3 of the 5 LSAs showed an increased overall survival when challenged with EL4 cells, as well as an increase in T cell frequency. Interestingly, two of our best candidates originated from an endogenous retroelement (ERE), which is a retroviral sequence that stably integrated the murine genome. This observation suggests that the strong immunogenic potential of those ERE-derived LSAs is caused by their ancestral viral origin.

Conclusion: Here, we developed the first proteogenomic platform able to identify any type of LSAs and demonstrated that targeting a single ERE-derived LSA prevents leukemia onset in mice. If this conclusion holds true in human, our study will help identifying and selecting the best antigens for leukemia as well as solid tumor immunotherapies.

PO15

Title: Podoplanin expression defines acute promyelocytic leukemia and contributes to fatal bleeding

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Affiliation: Guy Sauvageau, Médecine, IRIC, Université de Montréal

Keywords: Acute promyelocytic leukemia, marker, podoplanin, bleeding

Background information: Acute promyelocytic leukemia (APL) is a subgroup of acute myeloid leukemia (AML) characterized by the t(15;17) chromosomal translocation. APL is a medical emergency because of associated subgroup-specific lethal early bleedings, but the mechanisms underlying these complications are still not completely elucidated. Prompt diagnosis, which is often challenging, and urgent treatment with all-trans retinoic acid (ATRA) are required to decrease bleeding-related mortality.

Purpose of the study: We aimed to identify i) novel determinants of APL-related bleedings and ii) easily assessable APL-specific markers that could be used for rapid diagnosis.

Methods: RNA-sequencing of 30 APL, 400 AML and 66 normal samples was performed as part of the Leucegene project (Lavallée et al, Nature Genetics, 2015). Lentiviral vectors containing podoplanin (PDPN) or empty vectors were transduced in OCI-AML5 cells to engineer human leukemia cells expressing (AML5PDPN) and not expressing (AML5CTRL) PDPN, respectively. PDPN surface expression was assessed using PDPN-PE or PDPN-PE-Cy7 antibodies. Light transmission aggregometry was performed using two 2-channel Chronolog-Log 700 aggregometer. Cell culture and chemical screen experiments were performed as previously described (Lavallée et al, Nature Genetics, 2015). Sub-lethally irradiated NSG mice were transplanted with 2 million AML5CTRL or AML5PDPN cells. Mice platelet counts were serially assessed on the Yeti flow cytometer, and in vivo bleeding times were measured after amputation of the tail of anesthetized animals at 3 mm from the tip before sacrifice.

Results: Comparative RNA-sequencing analysis identified PDPN, which is involved in platelet aggregation during embryogenesis, as a transcript uniquely expressed in APL cells (median RPKM: 2.6 for APL vs 0 for AML, q-value = 7.3x10-29). Using a flow cytometry-based test, we established that PDPN represents the most specific surface marker in APL, and that ATRA treatment rapidly decreases its expression on primary APL cells in vitro. Functional studies showed that engineered overexpression of PDPN in human leukemic cells causes aberrant platelet binding, activation and aggregation, properties also observed in PDPN-expressing, but not in PDPN-negative, primary leukemias. Engineered PDPN expression on leukemic cells in a xenograft model was distinctively associated with thrombocytopenia and prolonged bleeding time in vivo.

Conclusion: PDPN represents a new APL-specific marker that could have important implications in early diagnosis and consequently on survival of APL patients. Aberrant PDPN expression on APL cells leads to decreased platelet counts and represents a novel determinant of APL-induced bleedings, paving the way for the design of novel therapeutic strategies for this disease.

PO16

Title: Modulation of T cell function by CD271

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Keywords: Mesenchymal stromal cells (MSC), graft-versus-host-disease (GVHD), CD271, T cells, immunoregulation.

Background information: Allogeneic hematopoietic stem cell transplantation is often the only curative treatment available for leukemic patients that have relapsed after initial treatment. One drawback of this treatment is the potential development of graft-versus-host disease (GVHD), a major cause of transplantation-related mortality. GVHD is mediated by donor T cells that recognize patient tissues as foreign, and standard immunosuppressive therapies targeting these T cells frequently fail to control this complication. Mesenchymal stromal cells (MSC) suppress T cell function and are being used with some success to treat GVHD in pediatric leukemia patients. CD271, a member of the TNFR superfamily that plays important roles in regulation of the immune system, is expressed on several immunomodulatory cell types including some mesenchymal stromal cell (MSC) preparations, but its role in T cell modulation is not known.

Purpose of the study: We seek to (1) investigate the immunoregulatory role of CD271 and (2) determine whether CD271 identifies MSCs with greater immunosuppressive potential.

Methods: First, T cell activation, proliferation and cytokine production have been assessed following in vitro stimulation of mouse and human T cells (with anti-CD3/anti-CD28) in the presence or absence of recombinant CD271-Fc protein. In addition, we are taking advantage of CD271-deficient mice to determine the proportion and activation state of immune cells in vivo in steady state as well as in autoimmune model in the absence of this protein. Finally, we are working to determine the extent to which immunomodulatory cell types, such as MSCs, isolated from WT and CD271-deficient mice can inhibit T cell activation in mixed lymphocyte reactions.

Results: Indeed, in vitro stimulation of mouse and human T cells in the presence of recombinant CD271-Fc protein inhibits T cell activation, proliferation and cytokine production. Further, although the proportion and activation state of immune cells are similar in adult CD271-deficient and control mice at steady-state, our preliminary data suggest that CD271-deficient mice have a more severe phenotype in autoimmune model as compared to control animals.

Conclusion: Together, these results suggest that CD271 modulates T cell function. We are working to determine the immunosuppressive or immune privilege properties of MSCs expressing CD271 to identify the feasibility of using this molecule as a biomarker for efficacious MSC preparations.

PO17

Title: PRMT5 is required for B cell proliferation and germinal center maintenance

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Keywords: Arginine Methylation, B cells, PRMT5, germinal center

Background information: After an infection, cognate antigens and T cell help activate mature B cells, inducing rapid B cell proliferation and the formation of germinal centers (GC). GC B cells undergo somatic hypermutation, underpinning affinity maturation, and isotype switching. The GC reaction requires the B cells to undergo major changes to their metabolic and cell cycle programs in order to proliferate at an extreme rate while undergoing programmed DNA damage. Arginine methylation is a post-translational modification that affects many cellular functions relevant to GC biology, but which is understudied in B cells. Purpose of the study) We want to study the role(s) of the protein arginine methyl transferase 5 (PRMT5) in GC B cells. Methods) Using mice in which PRMT5 is ablated in GC B cells we performed quantitative and qualitative analysis of their GCs after immunization and infection. Results) In vivo, eliminating PRMT5 results in GC collapse and completely blocks antigen-specific antibody production. The loss of GCs correlates with reduced intracellular Ki67 in GC B cells, suggesting PRMT5 is required for GC B cell proliferation. We confirmed a proliferation defect ex vivo by deleting Prmt5 in activated mouse primary B cells. This leads to cell cycle arrest in S-phase. We have excluded a potential role of DNA damage and replication stress in this arrest. Using RNAseq, we have identified novel perturbed pathways in Prmt5-/- B cells and are currently exploring their role in the GC reaction. Conclusions) Symmetrical arginine methylation by PRMT5 is a critical posttranslational modification regulating survival and cell cycle progression. As PRMT5 is overexpressed in B cell lymphomas, we hope to identify novel ways to target PRMT5 in malignant B cells by elucidating its role in GC B cells.

PO18

(absent)

Title: X-Linked Dyskeratosis Congenita Mutations affect the Dyskerin-Telomerase RNA Interaction

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Keywords: Dyskerin, Telomerase, Dyskeratosis Congenita, Bone Marrow Failure

Background: Defective telomere maintenance causes the premature aging disease dyskeratosis congenita (DC). DC is characterized by early onset of age-related maladies including bone marrow failure and increased risk of blood cancers like acute myeloid leukemia. The H/ACA ribonucleoprotein dyskerin is implicated in the most common form of DC (X-DC), and is essential for telomere maintenance. Our lab established that impairing dyskerin SUMOylation at disease-implicated lysine residues negatively affects levels of the H/ACA RNA used as a telomere synthesis template (hTR), leading to telomere shortening. Regulation of the telomere-synthesizing enzyme telomerase through dyskerin SUMOylation is not well understood mechanistically. Studying this post-translational modification of dyskerin will ameliorate our understanding of its roles in telomerase and telomere maintenance, as well as improve our knowledge of related disease phenotypes and treatment avenues.

Purpose: The objective of this project is to determine how SUMOylation of dyskerin regulates telomerase function in telomere syndromes. We will characterize disease-causing substitutions in dyskerin that affect SUMOylation and telomerase RNA biogenesis.

Methods: Two lysine residues implicated in premature aging disease were identified by mass spectrometry as SUMOylation sites. These SUMOylation sites fall outside of the putative RNA binding domain and have not been previously studied for RNA-binding effects. Dyskerin variants defective for SUMOylation at disease-implicated lysine residues were examined for RNA interactions by co-immunoprecipitation and qPCR. DC-causing variants of dyskerin previously reported to display RNA-binding deficiencies were also examined. Interactions between dyskerin and other H/ACA ribonucleoproteins were examined by co-immunoprecipitation from cell extracts to investigate effects of X-DC mutations on the H/ACA complex.

Results: DC-causing SUMO-defective dyskerin variants have impaired RNA interaction specific to hTR, offering a possible explanation for the previously observed decreased hTR accumulation in dyskerin knock-down cells expressing these variants, as well as a potential contributing cause of DC in patients harboring amino acid substitutions at these sites. Interactions of the H/ACA complex do not appear to be disrupted by these substitutions, supporting the notion that these X-DC mutations may disrupt telomerase specifically. Other X-DC causing mutations have varying effects on dyskerin-H/ACA RNA interactions beyond hTR, possibly contributing to the heterogeneity of X-DC presentation.

Conclusion: Telomerase regulation and telomere maintenance are connected to post-translational modifications of telomerase complex proteins like dyskerin. Analyzing the consequences of impaired dyskerin SUMOylation will lead to improved understanding of associated premature aging and blood disorders, their phenotypes, and potential for treatment. Our data suggest that X-DC-causing SUMO-defective variants of dyskerin have impaired RNA binding specific to hTR. In the future, RNA binding effects will be examined using dyskerin-SUMO fusion proteins previously shown to prevent loss of hTR. Telomere shortening caused by dyskerin knock-down was previously observed in our lab, and could not be rescued by introducing X-DC-causing SUMO-defective variants. We will investigate if imitating SUMOylation with fusion constructs of these variants can prevent telomere length maintenance defects.

PO19

Title: Lipid and lipoprotein abnormalities in acute lymphoblastic leukemia survivors

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Keywords: dyslipidemia, lipid and lipoprotein metabolism, acute lymphoblastic leukemia survivors, clinical studies, cardiovascular diseases.

Background information: Survivors of childhood acute lymphoblastic leukemia (cALL) are at risk of developing long-term cardiometabolic complications such as dyslipidemia, but the mechanisms explaining these late effects remain enigmatic. While it is well known that metabolic disturbances can markedly modify lipoprotein composition, the impact of disease and treatment on these processes is still unclear.

Purpose of the study: This study aims to examine the potential alterations in lipid profile and lipoprotein composition (VLDL, LDL, HDL2 and HDL3) in children and young adults recruited as part of the PETALE study at Sainte-Justine University Hospital (SJUH).

Methods: The lipid and lipoprotein profile was analyzed for ALL survivors (n=80) and healthy unrelated controls (n=22) matched for gender and age. Lipoprotein fractions were isolated by ultracentrifugation. The lipid and apolipoprotein (Apo) composition of each lipoprotein fractions were analysed by enzymatic tests and SDS-PAGE, respectively.

Results: Our results show that, despite their young age, 50% of cALL survivors displayed dyslipidemia, characterized by increased plasma triglycerides and LDL-cholesterol, and decreased HDL-cholesterol. cALL survivors exhibited lower plasma Apo A-I, higher Apo B-100 and C-II levels, along with elevated Apo C-II/C-III and B-100/A-I ratios. Compared to controls, VLDL fractions of dyslipidemic cALL survivors contained more triglycerides, free cholesterol and phospholipid moieties, but less protein. Differences in Apo content were found between cALL and controls for all lipoprotein fractions except for HDL3. Especially, HDL2 had reduced Apo A-I and raised Apo A-II, leading to a depressed Apo A-I/A-II ratio. Analysis of VLDL-Apo Cs disclosed a trend for higher Apo C-III1 content in dyslipidemic cALL survivors.

Conclusion: This comprehensive investigation demonstrates a high prevalence of dyslipidemia in cALL survivors while highlighting significant abnormalities in their plasma lipid profile and lipoprotein composition. Special attention must be paid to these subjects given the atherosclerotic potency of lipid and lipoprotein disorders.

PO20

Title: Identification of the molecular pathways mediating the anti-AML activity of statins

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Keywords: AML, statins, mevalonate pathway, therapy-resistance

Background information: Acute myeloid leukemia (AML) is the most common form of leukemia in adults with an overall survival of 26%. AML is divided into subgroups associated with good, intermediate and adverse outcome. We recently identified the ability of statins to discriminate between AML patient specimens from good and adverse outcome subgroups; specimens from good outcome patients were 10-times more sensitive to statins compared to those originating from adverse outcome patients. Statins are cholesterol-lowering drugs and work by inhibiting HMGCR, a rate-limiting enzyme of the mevalonate pathway. The discriminatory effect is lost with statin analog A405, synthesized by our team, and which lacks anti-HMGCR activity, suggesting that HMGCR is essential for the discriminatory effect of statins. However, A405 retains anti-leukemic activity towards a subset of therapy-resistant patients.

Purpose of the study: The mevalonate pathway comprises 7sub-branches; we therefore aim to determine which branch is responsible for the discriminatory activity of statins. Our second aim is to identify the target of statin analog A405.

Methods: In order to first validate the mevalonate pathway in statin-sensitive cell lines, we used RNAi to conditionally knock down the HMGCR gene and determined changes in statin sensitivity by viability assays. Once validated, we tried to genetically dissect the mevalonate pathway and its branches by using RNAi against key enzymes of the sub-branches. Lastly, in order to identify the target of the A405 analog, biochemical (pull-down assay) and genetic approaches (whole genome-CRISPR screen) will be undertaken.

Results: We achieved 50-80% HMGCR knockdown in statin-sensitive AML-5 cells with 5 different shRNAs against HMGCR. Our results showed that upon knockdown of HMGCR, AML-5 cells were rendered more sensitive to statins. Interestingly, although AML-5 cells were hyper-sensitized to statins, even after knocking down 80% of HMGCR, we did not observe a loss of viability suggesting that there could be pathways additional to the mevalonate pathway promoting leukemic effect in AML-5 cells. We therefore generated CRISPR knockout of HMGCR and observed that complete loss of HMGCR was lethal in AML-5 cells. Moreover, we were also able to rescue both the lethality of HMGCR knockout as well as the effect of statins with mevalonate, the downstream product of the HMGCR enzyme. We have also cloned shRNAs targeting the 7 branches in order to identify the main branch conferring sensitivity to statins.

Conclusion: Identifying the branch through which statins mediate their discriminatory effect will help identify pathways eliciting resistance in AML. Moreover, identifying the target of A405 will be key to improve the treatment of the subset of therapy-resistant AML patients sensitive to this compound.

PO21

Title: High-throughput characterization of transcription factor variants in the context of Acute Myeloid Leukemia

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Keywords: Acute Myeloid Leukemia, High-throughput, Transcription factors, Missense SNVs

Background information: Large scale sequencing projects over the last few years have identified a large number of novel mutations in the context of various diseases, including leukemia. Despite this, many patients lack recurrent mutations and the contribution of patient genetic background in these cases remains elusive. This phenomenon can be partially explained by the existence of large numbers of rare variants, or by the epistatic effect of certain combinations of mutations. Moreover, considering that each patient carries, on average, 100 damaging mutations in their genome, it would be virtually impossible to define the impact of each variant on pathogenesis, even with extremely large patient cohorts. Using bioinformatics tools, we uncovered and validated previously unknown damaging variants of GATA2, a key transcription factor in hematopoiesis and leukemia. Based on those results, we hypothesized that other known missense SNVs predicted to be damaging could also influence leukemia development.

Purpose of the study: We wish to identify and understand the specific contributions of those variants predicted to be damaging, in order to enhance our capacity to predict susceptibility to leukemia.

Methods and Results: We use transcription factors as a model system to characterize the impact of missense SNVs on protein function for their broad biological impact and practicality. The combined use of 2 bioinformatic tools has allowed us to predict the damaging effect of more than 400 missense SNVs in 78 transcription factors expressed in Acute Myeloid Leukemia, such as ETV6, SPI-1, HOXA6 and CEBPA. We are currently working to validate the impact of these mutations using a high-throughput luciferase reporter assay, by overexpressing either WT or variant transcription factors to compare their transcriptional activity. Those results will then be correlated with various parameters, such as transcription factor family, degree of conservation through evolution or gene function, to organize variants into patterns. We have cloned the majority of the 78 transcription factors and our preliminary results already highlight several novel damaging mutations. We are currently in the process of optimizing the assay for use in multiple cell lines to also show the influence of the expression of co-factors in SNV impact.

Conclusion: This study will not only allow us to validate the effects of the selected SNVs and perhaps improve the bioinformatics tools used for better predictions, but it will also help us understand how common and important those mutations are in the border context of leukemia in a controlled setting.

PO22

Title: Loss-of-function Mutations in SMARCD2 in Patients Suffering from Specific Granule Deficiency

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Keywords: Smarcd2, CEBPe, SWI/SNF complexes, chromatin remodeling, myelodysplastic syndrome

Background information: Homozygous CEBPe loss-of-function (LOF) mutations have been described in patients with neutrophil-specific granule deficiency (SGD), a rare autosomal recessive disorder characterized by defects in neutrophil function including atypical bilobed nuclei (pseudo–Pelgar-Huët anomaly), impaired migration and bactericidal activity and lack of both neutrophil and eosinophil secondary granule proteins. Although the full spectrum of clinical, cellular and molecular presentations of SGD remains unclear due to the rarity of the disease, most patients have decreased granulocyte and eosinophil numbers and evolve to a myelodysplasic syndrome. Though the first patients affected by this syndrome have been listed in the early 1970s, the molecular basis of this disease of the immune system is yet to be fully understood.

Purpose of the study: Recent observations from our lab suggest that specialized assemblies of SWI/SNF chromatin remodeling complexes regulate lineage determination in the hemopoietic tissue by providing distinct polymorphic surfaces for interaction with lineage-specific transcription factors. In particular, we showed that granulocyte development requires Smarcd2, a subunit of ATP-dependent SWI/SNF chromatin remodeling complexes. The purpose of this study was to investigate a potential role for the Smarcd2 subunit of SWI/SNF chromatin complexes in SGD.

Methods and Results: Using a conditional knockout allele, we showed that Smarcd2 is essential to generate functionally mature neutrophils and eosinophils. Notably, Klein and colleagues reported loss-of-function mutations in SMARCD2 in patients suffering from SGD, thus demonstrating that the function of this gene is maintained in humans. These patients suffer from severe infections due to the lack of neutrophils and eventually develop a myelodysplastic syndrome that often evolves into leukemia. We showed that Smarcd2 does not bind DNA but interacts with the CEBPe in hemopoietic cells. Using chromatin immunoprecipitation (ChIP) experiments, we demonstrated that Smarcd2 is necessary for CEBPe transcription factor recruitment to the promoter of neutrophilic secondary granule genes and for granulocyte differentiation. The highly homologous Smarcd1 protein (63% identical at the amino-acid level) cannot replace Smarcd2's role in granulocyte development. We find that Smarcd2 functional specificity is conferred by its divergent coiled-coil 1 and SWIB domains. Strikingly, both CEBPe and SMARCD2 loss-of-function mutations identified in SGD patients abolish the interaction with SWI/

SNF and thereby secondary granule gene expression, thus providing a molecular basis for this disease.

Conclusion: In summary, these studies led to the discovery of a critical regulator of the innate immune response and provide a better understanding of the molecular defects responsible for a disease of the immune system. Further characterization of SMARCD2 and CEBPe genetic defects and functional abnormalities will help to define their roles in human myelopoiesis and innate immunity and may eventually lead to the development of more effective therapeutic approaches for patients with SGD.

PO23

Title: Exploring the role of the KDM4A Jumonji-C demethylase at transcriptional enhancers in pediatric acute myeloid leukemia

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Keywords: lysine demethylase KDM4A, MLL-AF9 acute myeloid leukemia, transcriptional enhancer, bioinformatics, pharmacological inhibitors

Background information: Epigenetic modifications modulate the regulation of gene expression, cell cycle, genome stability and nuclear architecture. The numerous epigenetic modifiers that catalyze these reactions are currently under intense study as they have been implicated in many cancers and human diseases. The KDM4A/JMJD2A lysine demethylase mediates the removal of methyl groups from di- or tri-methylated lysines 9 and 36 of histone H3 (H3K9 and H3K36). A role for KDM4A as an epigenetic regulator at transcriptional start sites (TSS) is now well established. Through chromatin immunoprecipitation (ChIP) followed by deep sequencing (ChIP-seq) analysis, we have identified the localization of KDM4A at many non-TSS genomic loci that are enriched in epigenetic modifications characteristic of transcriptional enhancer regions, which are the drivers of cell-type and context-specific gene expression. We hypothesize that KDM4A modulates transcriptional programs by binding to genomic enhancers in a context-dependent manner.

Purpose of this study: The principal aim of this study is to decipher the role for KDM4A at novel genomic enhancer targets in acute myeloid leukemia (AML), where KDM4A is overexpressed and known to contribute to leukemic cell maintenance. An important focus of our research is to explore KDM4A as a target for the development of novel pharmacological therapies.

Methods: Using molecular (shRNA) and pharmacological inhibition methods followed by whole-transcriptome and ChIP-sequencing, we are investigating the precise role of KDM4A at enhancers in leukemic cells carrying the MLL-AF9 fusion and other cancer cell lines. We have analyzed our sequencing data using a variety of bioinformatics approaches and integrated these results with publicly available data sets to predict novel transcriptional targets of KDM4A.

Results: Our preliminary results suggest that KDM4A localizes to active and biologically relevant enhancer regions. Sequence motif analyses of KDM4A-bound enhancer loci revealed that these regions are also enriched for transcription factor binding motifs. Interestingly, the binding of KDM4A to enhancers was not accompanied by a modulation of levels of H3K9me3/H3K36me3, which suggests that this event occurs independently of the catalytic demethylase activity of KDM4A depletion. Strikingly, the depletion and inhibition of KDM4A leads to growth arrest and cellular senescence in mouse and human leukemic cell lines.

This phenotype was accompanied by the transcriptional modulation of several genes involved in MLL-AF9 leukemogenesis, as determined by quantitative PCR. We have also identified the molecular interaction and genomic co-localization of the well-known leukemic-cell required BAF chromatin remodeling complex with KDM4A.

Conclusion: Our study will provide fresh insight into a role for KDM4A at genomic enhancers and novel information about the function of this epigenetic regulator in pediatric AML, a disease still plagued with dismal survival rates. Our results demonstrate that KDM4A inhibition and depletion leads to the growth arrest of MLL-AF9 leukemic cells in vitro. Our ongoing experiments will include a study to better understand how the epigenetic and enhancer landscapes are differentially modulated by KDM4A in normal versus leukemic stem cells, as well as a characterization of the interaction of KDM4A with the BAF chromatin remodeling complex in the MLL-AF9 leukemia genome.

PO24

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

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Keywords: EZH2, PRC2, Leukemia, Epigenetic, Hematopoiesis

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

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

Methods: We recently generated an Ezh2/Ezh1 (Ezh1/2) conditional double KO (DKO) mouse model to completely abrogate the catalytic activity of the PRC2 complex in hematopoietic cells. We used competitive transplantation assays to evaluate the self-renewal and the proliferation potential of transgenic hematopoietic stem cells in WT, p53-/- or p16/p19-/- genetic backgrounds. We also performed rescue-type experiments with mutant Ezh2 constructs to determine which domains are essential to its function. We also used histone mutants in MEFs cells to determine if the observed phenotypes were solely due to a loss of histone methylation. Finally, we generated HoxA9-Meis leukemia and evaluated the impact of PRC2 inactivation on disease development.

Results: While Ezh1 or Ezh2 inactivation alone does not induce a dramatic effect, concomitant inactivation of Ezh1/2 in the blood system rapidly leads to aplastic anemia and bonne marrow failure. Competitive assays have shown that hematopoietic stem cell originating from double KO mice are not able to compete with their wild type counterparts and will rapidly disappeared from the recipient animal. Re-introduction of wild type Ezh2 but not a catalytic dead mutant is able to completely restore cell survival. Ezh2 inactivation leads to a strong de-repression of p19 but inactivation of the In4A/Arf p53 loci is not able to restore cell survival suggestion additional mechanisms. Overexpression of a dominant negative H3K27M histone mutant only partially phenocopies PRC2 inactivation Finally, Ezh1/2 co-inactivation greatly delay the HoxA9 Meis1 leukemia onset in comparison with Ezh2 inactivation alone, while

Ezh1 inactivation do not give any advantages compared to WT HoxA9 Meis1 leukemia.

Conclusion: Together, these studies show that Ezh1/2 are essential regulator of normal and leukemic hematopoietic stem cells, likely through their methyltransferase activity. We demonstrated that p16/p19 de-repression can't explain the observed phenotypes and suggestion other mechanisms. Moreover, our results showed that part of the phenotype is independent of H3K27 methylation defects and suggest that EZH2 could have important non-histones targets. In conclusion EZH1 and EZH2 share redundant functions and could be promising therapeutics targets.

PO25

Title: Optimizing the T cell repertoire for adoptive immunotherapy

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Keywords: adoptive T cell therapy, immunotherapy, CD4 T cells, CD5, interferon-gamma

Background: Adoptive T cell therapy has emerged as a promising treatment for patients who are otherwise refractory to traditional therapies. Unfortunately, a large portion of treated patients relapse, and these relapses correlate with acquired mutations in the target antigen or an inability to detect the transferred T cells in vivo. Typically, total T cell pools are used as a source for these therapies. However, recent evidence suggests that within naïve T cell populations, there is significant heterogeneity in terms of their differentiation potential and effector functions that could directly impact treatment outcome. Consistent with this, CD5, a negative regulator of T cell receptor signaling, can be used to enrich for murine T cell populations that respond differentially during antigen challenge.

Purpose of the study: The goal of this project is to characterize the functional heterogeneity of naïve T cells based on their CD5 expression and thus identify a T cell subset that would be more effective for adoptive therapy.

Methods: To evaluate helper T cell differentiation biases among T cells, naïve CD4 T cells from murine splenocytes were sorted based on their CD5 expression (top and bottom 20%) and activated under non-skewing or T helper 1 (Th1) skewing conditions in vitro. Cytokine production and transcription factor expression were analyzed by flow cytometry. To complement our in vitro analysis, we infected mice with LCMV and assessed interferon-gamma (IFNγ) production by antigen specific CD5-hi and CD5-lo CD4 T cells. Given the recent evidence that glycolysis positively regulates IFNγ production, we evaluated the ability of CD5-hi and CD5-lo cells to engage glycolysis by flow cytometric analysis of glucose transporter 1 (Glut1) and phospho-S6, two markers associated with increased glycolytic capacity.

Results: Our data shows that ex vivo activated murine CD5-lo CD4 T cells produce relatively greater amounts of the Th1 cytokine IFNγ compared to their CD5-hi counterparts. This difference is specific to IFNγ and is independent of expression of the Th1 specific transcription factor, T-bet. Consistent with the in vitro analysis, our preliminary in vivo data suggests that during acute LCMV infection, antigen specific CD5-lo CD4 T cells produce more IFNγ than their CD5-hi counterparts upon antigen-specific re-stimulation. Unexpectedly, our data point towards a cellular metabolism-independent mechanism of regulation of IFNγ production as CD5-hi cells express higher levels of Glut1 and phospho-S6.

Conclusion: Our data provides new insights into intrinsic functional differences within the naïve CD4 T cell population. Given the importance of CD4 T cells in prolonging therapeutic T cell responses and IFNγ in inducing epitope spreading, we now seek to determine whether CD5-lo CD4 T cells can augment the durability and efficacy of adoptive T cell therapies.

PO26

Title: Phosphoproteomic and genetic analysis of UM171-treated hematopoietic stem and progenitor cells

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Keywords: Graft optimization, hematopoietic stem and progenitor cells, ex-vivo expansion, UM171.

Background information: Hematopoietic stem and progenitor cell (HSPC) transplantation constitutes one of the most effective therapeutic strategies for patients with leukemia. HSPC are able to restore a healthy bone marrow through their ability to sustain lifelong production of all mature blood cells in vivo. Human cord blood (CB) transplantation is ideal to this purpose, thanks to its immature immune system and rapid availability. However, as most CB grafts suffer from low cell numbers, expansion of HSPC becomes of great interest. Our lab has developed optimized culture conditions to expand fully functional HSPC from human CB. In particular the small molecule UM171 is able to expand primitive cells in culture and represent, therefore, an important tool to understand the molecular basis for HSC self-renewal and differentiation and translate this know how to bone marrow transplantation clinics.

Purpose of the study: The aim of this research project is to use phosphoproteomic and transcriptomic approaches to uncover cellular pathways essential to HSC self-renewal and differentiation and to identify new extracellular markers to allow HSC isolation in culture.

Methods and Results: Signaling pathways rapidly activated by UM171 and connected to the expansion and differentiation of primitive CB-derived CD34+ cells have been evaluated by means of a phospho-proteomic approach by stable isotope labeling with amino acids in culture (SILAC). Lysates from cells treated with UM171 or the vehicle at specific time points have been proteolytically digested and analyzed by LC-MS/MS. Through this approach we were able to point out that UM171, beside its known effect in HSC expansion, is also able to expand progenitors of several immune cell subtypes, especially mast cells and dendritic cells.

Moreover, in an attempt to improve the accuracy of prospective HSC identification in culture, we performed several transcriptomic analysis of UM171-treated CB cells, which allowed the identification of 2 new surface markers: EPCR (Endothelial Protein C Receptor) and ITGA3 (Integrin α3). CB cells expanded for 7 days in presence of UM171 were transplanted into NSG mice, and showed that the expression of ITGA3 is sufficient to split the highly primitive CD34+CD45RA-CD90+EPCR+ population in two functionally distinct subpopulations presenting short-term (ITGA3-) and long-term (ITGA3+) repopulating abilities.

Conclusions: Overall, our results allowed a better comprehension of UM171's ability to expand hematopoietic stem cells and immune cells progenitors. Our data not only will expand our knowledge about the biology of hematopoietic stem cells but might also lead to the production of optimized cord blood grafts which will be readily translated to the clinic.

PO27

Title: BH3 Profiling of Primary Non-Hodgkin Lymphoma Samples

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Keywords: Non-Hodgkin Lymphoma, Apoptosis, BH3 profiling, Personalized Medicine, Translational Research

Background information: Lymphomas are the third most common cancer in the adolescent and young adult population (18-39 years old), of which diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma (BL) are the most common non-Hodgkin lymphomas (NHL). These are very aggressive lymphomas that can be cured with multi-agent chemotherapy, however, relapsed lymphoma is fatal in 90% of cases. A major cause of mortality in these patients is the co-expression of two proteins, MYC and BCL2, which together synergize to promote cellular proliferation and inhibition of apoptosis. A new drug called venetoclax targets BCL2 and holds promise to improve the survival of these patients. However, it is active in only a subset of BCL2 producing lymphomas. Understanding which patients respond to venetoclax and how to increase the proportion of responders will result in better treatment options for this group of patients.

Purpose of the study: We propose to study the apoptotic blocks in primary lymphoma patient samples using a "BH3 profiling" assay, which determines the cells' dependence on specific anti-apoptotic proteins for their survival and thus predicts their readiness to be killed by chemo-therapy3. By understanding the apoptotic status of the tumors, this technique may be able to guide the selection of the chemotherapy likely to be the most effective against that specific cancer.

Methods: BH3 profiling specifically measures mitochondrial outer membrane polarization (MOMP), using cytochrome c release as a marker, measured by flow cytometry, after exposure to synthetic pro-apoptotic BH3 peptides. These synthetic BH3 peptides each have their own affinities for anti-apoptotic proteins that may be blocking the cell's ability to undergo apoptosis. If exposure to the synthetic peptide is able to cause MOMP, we can conclude that the anti-apoptotic protein that it targeted was blocking the cell's ability to undergo apoptosis. We perform this assay on both untreated cells (baseline profiling) in addition to cells that have been pre-treated with various chemotherapies (dynamic profiling) to determine if and how the treatment alters the profiles, and whether or not the cells' sensitivies to venetoclax are increased.

Results: We have performed baseline profiling on 57 primary samples and have seen great heterogeneity across samples. CLL samples are the most 'primed' group, and have about a 50% baseline response to venetoclax. FL samples are also quite primed as a group, but have a much lower (15%) baseline response to venetoclax. The DLBCL sample group is much less primed overall than the FL and CLL groups, and has very low responses to venetoclax (6%). We have also tested cell lines, and have found that venetoclax-insensitive cells can become sensitive after pre-treatment with Doxorubicin or Vincristine.

Conclusion: Non-hodgkin Lymphoma is an extremely heterogeneous disease, with varying levels of baseline sensitivity to the BCL-2 inhibitor venetoclax. We believe that pre-treatment with components of the R-CHOP treatment regimen may increase the sensitivity to venetoclax, and have shown this to be true in cell lines. We are beginning to test this hypothesis in primary samples.

PO28

Title: Role of the multidomain epigenetic regulator BRPF1 in leukemia development

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Keywords: leukemia, histone, acetylation, MOZ-TIF2, BRPF1

Background information: The monocytic leukemia zinc finger protein (MOZ) is a histone acetyltransferase firstly identified in a recurrent translocation t(8;16)(p11;p13) with fusion partner of CBP (CREB-binding protein) leading to an acute myeloid leukemia (AML) (Borrow, J., et al., Nat. Genet., 1996). The MOZ gene is also rearranged in AML with the chromosome inversion inv(8)(p11;q13), generating MOZ-TIF2 (transcription intermediary factor 2) containing N-terminal part of MOZ including catalyzing MYST domain and C-terminal part of the TIF2 (Liang, J., et al., Blood, 1998). It has been shown that MOZ is required for maintaining the self-renewal of hematopoietic stem cells (HSCs). Moreover, aberrant fusion protein MOZ-TIF2 confers leukemic stem cell (LSC) properties such as self-renewal to committed hematopoietic progenitors and consequently promotes leukemia development (Huntly, B.J., et al., Cancer Cell, 2004). We previously demonstrated that MOZ-TIF2 fusion protein forms complex with an epigenetic regulator BRPF1. BRPF1 acts as a scaffold protein of the MOZ complex and functions as an epigenetic regulator to stimulate acetyltransferase activity, restrict substrate specificity and potentiate transcriptional ability of wild-type MOZ. This may also apply to leukemic proteins such as MOZ-TIF2 because of its retention of the catalyzing domain of MOZ for BRPF1 binding.

Purpose of the study: We have recently reported that BRPF1 is essential for mouse hematopoietic stem cells and histone H3K23 acetylation (H3K23ac) significantly reduces in BRPF1 KO MEFs and spleen (Yan, K. et al., Am. J. Hum. Genet., 2017), but it remains unclear whether BRPF1 regulates the unique ability of MOZ-TIF2 to confer LSC properties such as self-renewal to committed progenitors. So my research interest is to investigate whether and how BRPF1 regulates leukemic activities of MOZ-TIF2 in vitro and in vivo.

Methods: In vitro histone acetylation assays were used for examining the H3K23 acetylation ability of MOZ-TIF2 and wide type MOZ, regulated by BRPF1. In addition, we will employ colony-formation assays with transduction of MOZ-TIF2 in mouse bone marrow cells to determine whether BRPF1is required for MOZ-TIF2 to confer self-renewal to committed progenitors. In vivo, we will perform bone marrow transplantation assays to assess how BRPF1 collaborates with MOZ-TIF2 to induce LSC properties.

Results: In vitro histone H3K23 acetylation assays showed that N-terminal part of MOZ including the catalyzing MYST domain is sufficient for H3K23 acetylation. This was also observed the remained H3K23ac level in a C-terminal region of MOZ deleted mice (Sheikh, B. et al., Oncogene, 2015). Moreover, we also found that the N-terminal to argine-455 of BRPF1 is required for MOZ interaction and H3K23 acetylation. We will analyze how MOZ-TIF2 interacts with BRPF1 in vitro and in vivo by using histone acetylation assays, colony-formation assays, and bone marrow transplantation assays.

Conclusion: Results from this study will shed light on how the novel epigenetic regulator BRPF1 regulates leukemia development induced by MOZ-TIF2 and other related leukemic fusion proteins.

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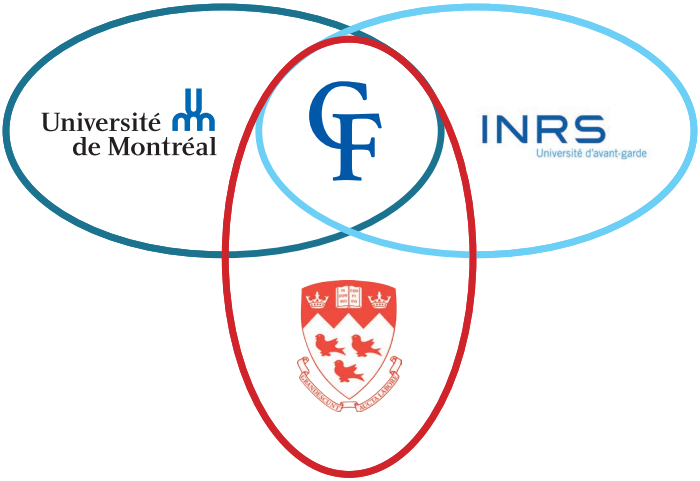
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