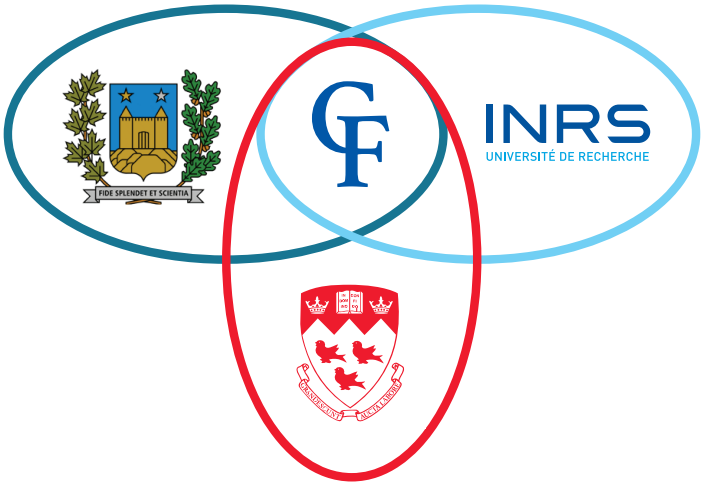
12th YEAR

Cole Foundation
"Research Celebration Day"

MAY 11, 2018



12ième ANNÉE

Journée
« Célébrons la recherche »
de la Fondation Cole

11 MAI 2018

THE COLE FOUNDATION

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD programmes to promote research inpreleukemia, 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 2018 Fellows, the Fellowship programme has supported more than 175 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 establishment of the Jack Cole Chair in Pediatric Oncology and Hematology at McGill University.

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

THE COLE FOUNDATION

Mr. Barry Cole – President
Mr. John Moran – Secretary/Treasurer
Mr. David Laidley – Board Member
Ms. Anne Lewis – Board Member
Mr. Bruce McNiven – Board Member
Dr. Morag Park – Board Member
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Ms. Gabrielle Cole – Board Member
Ms. Viviane Cole – Board Member
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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 2018, le programme a appuyé plus de 175 chercheurs répartis dans des laboratoires et des hôpitaux de l'agglomération montréalaise, à la faveur de collaborations avec l'Université de Montréal, l'Université McGill et le Centre INRS – Institut Armand-Frappier. Plus de sept millions de dollars y ont été consacrés.

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

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

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

PROGRAM

9:30 - 10:00 AM	Round Table (new fellows) Holmes Hall, 3605 rue de la Montagne
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 Martin Amphitheater, McIntyre Building
Welcome	***Dr. Elaine Davis*** *Associate Dean, Biomedical BSc, Graduate and Postdoctoral Affairs, McGill University*
New Research - New Realities Presentations	***Dr. Ivan Litvinov*** *MUHC Research Centre* *« Ectopic expression of meiomitosis genes and reactivation of LINE-1 retrotransposons in Cutaneous Lymphomas»*
	Dr. François Mercier *Lady Davis Research Institute* *« Dissecting leukemic stem cell function using the CRISPR toolbox»*
	Dr. Thai Hoa Tran *CHU Sainte Justine* *« Ph-like ALL : translating genomic discoveries into optimized therapies»*
2:00 - 3:15 PM	Introduction ***Dr. Kolja Eppert*** *Assistant Professor, the Department of Pediatrics, McGill University*
Keynote Speaker	***Dr. Soheil Meshinchi*** *Full Member, Fred Hutchinson Cancer Research Center Professor of Pediatrics, University of Washington School of Medicine* ***« Genome and Epigenome of AML in Children and Young Adults»***
3:15 - 3:30 PM	New fellows and announcement of prizes
3:30 PM	Reception

THE 2018 – 2020 COLE FOUNDATION FELLOWS

MCGILL UNIVERSITY

Patricia Arreba Tutusaus, Post PhD program
Supervisor: Kolja Eppert, McGill University Health Centre, Pediatric Hematology-Oncology
Project title: Intercellular communication in myeloid malignancies – Uncovering novel mechanisms to target leukemic stem cells

Description: In acute myeloid leukemia (AML), disease-initiating leukemic stem cells (LSCs) are shielded by the bone marrow, which supports their growth and protects them against chemotherapy. This project aims to uncover the reciprocal communication between LSCs and their environment through unique cellular components know as exosomes.

Jennifer Ganchev, PhD program
Supervisor: Ivan Litinov, Lady Davis Research Institute
Project title: Investigation of moleculer lymphomagenesis and novel pathways of genomic instability in hypopigmented mycosis fungoides

Description: We have recently shown the ectopic expression of meiotic germ cell genes and the reactivation of LINE-1 retrotransposition in CTCL. The aim of this project is to elucidate the role of genomic instability attributed to these processes in skin and systemic pediatric lymphomas

John Heath, PhD program
Supervisor: Alexandre Orthwein, Lady Davis Research Institute
Project title: Deciphering the role of the zinc finger protein POGZ in B cell diversification and pediatric Burkitt Lymphoma

Description: We have identified a novel role of REV7 interactor, POGZ, in modulating DNA damage responses and B cell maturation. Therefore, my project aims are to identify how POGZ regulates genome integrity, in both B cell diversification and lymphoma pathology in vivo, and to validate these findings and explore how POGZ impacts responses to lymphoma therapy in transgenic mouse models.

Yun Hsiao Lin, PhD program
Supervisor: Anastasia Nijnik, McGill University, Department of Physiology
Project title: MYSM1 as a potential therapeutic target for c-MYC-driven hematological malignancies

Description: This project aims to elucidate the molecular mechanisms underlying the function of deubiquitinase MYSM1 in c-MYC-driven lymphoma/leukemia. As abnormal expression of c-MYC is often associated with pediatric and young adolescent B-cell lymphoma/leukemia, we hope this work will contribute to the understanding and treatment of c-MYC-driven hematological malignancies.

LES BOURSIERS DE RECHERCHE DE LA FONDATION COLE 2018 – 2020

Poorani Ganesh Subramani, PhD program
Supervisor: Javier Di Noia, McGill University, Department of Microbiology & Immunology
Project title: Characterization of factors determining physiological and oncogenic mutagenesis by AID in B cells

Description: To generate effective high-affinity antibodies, Immunoglobulin genes in activated B-cells must be mutated by Activation Induced Cytidine Deaminase (AID). Collaterally, AID can also mutate other "off-target" genes, leading to tumorigenesis. We recently identified a group of novel AID interactors and we aim to characterize how these interactors modulate physiological and oncogenic mutagenesis by AID.

UNIVERSITÉ DE MONTRÉAL

Léo Aubert, Post PhD program
Supervisor: Philippe Roux, IRIC, Pathology and Cell Biology
Project title: Define the surfaceome landscape of hematopoietic stem cells and pediatric leukemia specimens to improve the development of novel therapies for hematological diseases

Description: This collaborative multidisciplinary project is part of a main program, which aims to improve therapies of pediatric patients with leukemic disorders through the optimization of cord blood grafts or through the development of antibody-based therapies.

Héloïse Chassé, Post PhD program
Supervisor: Katherine Borden, IRIC, Pathology and Cell Biology
Project title: A novel regulative function of the eukaryotic translation Initiation Factor 4E in cancer cells: Control of m6A-methylome dynamics

Description: This project aims to determine if there are functional regulations occurring between the oncoprotein eIF4E and the m6A epitranscriptomic marks dynamics. Both are commonly dysregulated in AML thus altering the proteome without altering transcription. Studying these processes together will allow a better understanding of the fundamental mechanisms contributing to the oncogenic phenotype of AML cells.

Margaret Davis, Post PhD program
Supervisors: Katherine Borden, IRIC, Pathology and Cell Biology
Project title: Novel functions of eukaryotic translation initiation factor 4E (eIF4E) in leukemia

Description: This project aims to define the novel role of eIF4E in 3'processing. Further, the research will establish new insight into RNA processing and allude to alternate regulatory pathways within adult and pediatric leukemia and lymphoma.

Gregory Ehx, Post PhD program
Supervisors: Claude Perrault, IRIC, Immunobiology
Project title: The molecular landscape of leukemia-specific antigens

Description: Our project aims at developing a vaccine which would be used to treat, not to prevent, acute myeloid leukemia (AML). To accomplish this, we have developed a proteogenomic pipeline which could allow us to identify novel molecules specifically expressed by AML cells and which could be targeted by a vaccine.

Aditi Sood, Post PhD program
Supervisors: Heather Melichar, Maisonneuve-Rosemont Hospital
Project title: Optimizing the T cell repertoire for T-cell therapy for leukemia

Description: T cell therapy has emerged as a promising treatment for pediatric leukemia. However, a major challenge to durable patient responses is a lack of T cell persistence and function in vivo. Having recently characterized T cell subsets with differential persistence and functionality, we aim to identify a T cell repertoire that will improve the efficacy of this therapy.

Aurelien Colamartino, PhD program
Supervisors: Elie Haddad, CHU Sainte-Justine
Project title: Nouvelle approche pour l'untlisatiohn des CAR dans la leucémie aigue lymphoblastizue de l'enfant

Description: The chimeric antigen receptor (CAR) therapy using T cell is a major improvement in the actual treatment of acute lymphoblastic leukemia. Despite the tremendous success, some patient still relapse. These relapses are linked to exhaustion of T cells. To overcome this issue we are developing a CAR therapy using hematopoietic stem cell transduced with a new synthetic promoter to direct CAR expression exclusively on T cell progeny.

Reece Dowling, PhD program
Supervisors: Denis-Claude Roy, Maisonneuve-Rosemont Hospital Research Centre
Project title: Targeting hematological cancers using TCR gene therapy

Description: T cell receptor (TCR) gene therapy presents an exciting new avenue for haematological cancer treatment, with encouraging pre-clinical results. The TCR is responsible for recognizing tumour antigens bound by human leukocytic antigen (HLA) molecules, were with co-stimulation allows a cytotoxic response. The aim of this project is to develop a novel TCR gene product targeting acute leukaemia's, which can be transduced into activated T cells, thus allowing a robust cytotoxic response against cancerous cells without host toxicity.

Gabrielle Duhamel, PhD program
Supervisors: Daniel Curnier, Université de Montréal, Département de kinésiologie
Project title: Feasibility of integrating a three-part program: physical activity, nutrition and psychosocial, at The Charles Bruneau Fondation Center at CHU Sainte-Justine

Description: Close to 75% of pediatric cancer survivors will develop chronic or late health issues. The practice of physical activity during oncologic treatments has the potential of increasing/maintaining physical capacities, have direct impact on health, QoL and potentially to show improvements in various health markers directly linked to the disease.»

Julie Laniel, PhD program
Supervisors: Sarah Lippé, CHU Centre de recherche Sainte-Justine
Project title: Les biomarqueurs cérébraux et génétiques des séquelles neurocognitives à long terme chez les survivants deleucémie lymphoblastique aiguë pédiatrique

Description: : Le projet vise à clarifier la présence des altérations de la substance blanche (SB) chez les survivants de LLA, étudier leur impact sur la performance cognitive, et ultimement à proposer un modèle combinant des facteurs génétiques et de l'intégrité de la SB pour expliquer l'apparition de ces séquelles cognitives tardives.

Thomas Milan, PhD program
Supervisors: Brian Wilhelm, IRIC, Medecine
Project title: Integrative epigenomic analysis to identify potential biomarkers in MLL-AF9 acute leukemia

Description: This project aims to better understand the biology of acute leukemias involving the MLL-AF9 fusion that is commonly found in pediatric leukemias. By using a human model leukemia, we are performing a genome-wide analysis to highlight epigenetic mechanisms involved in MLL-AF9 leukemogenesis.

Victor Oswald, PhD program
Supervisors: Philippe Robaey, CHU Centre de recherche Sainte-Justine
Project title: Étude de la connectivité fonctionnelle au repos chez le sujet normal et chez les survivants de la leucémie suite une intervention neurotoxique au cours du développement cerebral

Description: Les survivants de la leucémie démontrent des troubles cognitifs suite au traitement pour prévenir les rechutes méningées. En utilisant la magnétoencéphalographie, nous allons définir des marqueurs cérébraux qui distinguent les survivants avec ou sans séquelles cognitives 15 ans après le diagnostic de contrôles sains.

Lucas Poncelet, PhD program
Supervisors: Daniel Sinnett, CHU Centre de recherche Sainte-Justine
Project title: Development of a microfluidic device for the quantification of non-coding RNAs from whole blood aimed at the diagnosis and prognosis of childhood acute lymphoblastic leukemia

Description: Design an all-in-one automated lab-on-a-chip to isolate and purify exosomes from cALL patients' blood samples, extract their RNA content and quantify targets of interest. This tool will have the potential to be used directly by clinicians do detect biomarkers at the bedside of the patient.

Pierre Priam, PhD program
Supervisors: Julie Lessard, IRIC, Chromatin Structure and Stem Cell Biology research unit
Project title: SMARCD1 is Essential for Normal B cell Development and Lymphomagenesis

Description: We identified SMARCD1 as an essential gene for B cell production and lymphomagenesis through regulation of key factors involved in B cell-differentiation. In this work, we propose to dissect the mechanisms through which SMARCD1 regulates B cell lymphomas. These studies should lead to a better understanding and management of diseases involving B cell dysfunction.

Mathieu Roussy, PhD program
Supervisors: Sonia Cellot, CHU Sainte-Justine Research Centre
Project title: Engineering human models of high fatality pediatric leukemia induced by NUP98 fusions to identify novel biomarkers and functional susceptibilities

Description: Pediatric acute myeloid leukemia (AML) is a rare and heterogeneous disease, still plagued with low cure rates. We will generate synthetic human models of high fatality NUP98 rearranged AML using cord blood cells. The engineered cell lines and xenograft models will serve to identify novel disease biomarkers and drug targets.

COLE FOUNDATION POSTER SESSION
SESSION D'AFFICHES DE LA FONDATION COLE

PRESENTERS / LISTE DES EXPOSANTS

Rachid Abaji
Yahya Benslimane
Aubrée Boulet-Craig
Margaret Davis
Ema Flores-Diaz
Ryan Killoran
Vincent-Philippe Lavallée
Deana MacNeil
Mathiew Neault
Guillaume Richard-Carpentier
Aditi Sood
Elisa Tomelinni

Léo Aubert
Meaghan Boileau
Willow Burns
Marion Dubuissez
Martin Karam
Shirin Lak
Charles-Étienne Lebert-Ghali
Abba Malina
Linnea Olofsson
Christina Sawchyn
Jutta Steinberger
Claudia Wever

Alexandre Benoit
Karine Boulay
Elodie Da Costa
Heather Duncan
Fida Khater
Céline Laumont
Vincent Luo
Sophia Morel
Pierre Priam
Laura Simon
Daméhan Tchelougou
Ke Zhi Yan

PHD POSTER LIST / LISTE DES PROJETS EXPOSÉS DES DOCTORANTS

P01	Abaji, Rachid (2016-18)	Characterization of the functional impact of polymorphisms associated with asparaginase complications identified by exome-wide association study
P02	Benoit, Alexandre (2017-19)	The Role of mutated Ras-associating proteins in relapse/ refractory GCB-DLBCL
P03	Benslimane, Yahya (2016-18)	CRISPR-based genome-wide knockouts to identify synthetic lethality with telomerase inhibition in acute lymphoblastic leukemia
P04	Boileau, Meaghan (2017-19)	Leukemic stem cell expression signatures identify novel therapeutics for acute myeloid leukemia
P05	Boulet-Craig, Aubrée (2016-18)	Atypical visual short-term memory activation patterns in acute lymphoblastic leukemia survivors: A MEG study
P06	Burns, Willow (2016-18)	Cancer-related effects on relationships, long-term psychological status in couples whose child was treated for leukemia: A PETALE study
P07	Da Costa, Elodie (2017-19)	Oncogenic context determines therapeutic and epigenetic efficacy of cardiac glycosides in paediatric leukemia
P08	Duncan, Heather (2016-18)	G Protein-Coupled Receptors 56 and 114 as Potential Functional Regulators of Normal and Leukemic Human Stem Cells
P09	Flores-Diaz, Elodie (2017-19)	Targeting key vulnerabilities of pre-Leukemic Stem Cells in T-cell Acute Lymphoblastic Leukemia

P10	Karam, Martin (2017-19)	Deciphering the role of the zing finger protein POGZ during hematopoiesis and pediatric Acute Myeloid Leukemia development
P11	Lak, Shirin (2017-19)	Modulation of co-signaling to improve ex vivo human antigen-specific T cells generation for immunotherapy
P12	Laumont, Céline (2016-18)	Non-coding regions are the main source of tumor-specific antigens
P13	Luo, Vincent (2017-19)	OTUB1 in B cell development
P14	MacNeil, Deana (2016-18)	X-linked dyskeratosis congenita mutations disrupt the dyskerin-telomerase RNA interaction
P15	Morel, Sophia (2016-18)	Dyslipidemia in pediatric acute lymphoblastic leukemia survivors: a role for gut microbiota
P16	Priam, Pierre (2016-18)	Smarcd1 Subunit of SWI/SNF Complexes is Required for Normal and Pathogenic Lymphopoiesis
P17	Sawchyn, Christina (2016-18)	Exploring the role of the KDM4A Jumonji-C demethylase at transcriptional enhancers in pediatric acute myeloid leukemia
P18	Simon, Laura (2017-19)	The master transcription factor RUNX1 antagonizes the Glucocorticoid Receptor pathway in Acute Myeloid Leukemia
P19	Tchelougou, Daméhan (2017-19)	A novel mechanism of regulation of the tumor suppressor BAP1/ASXL2 complex by monoubiquitination
P20	Yan, Ke Zhi (2016-18)	Role of the multidomain epigenetic regulator BRPF1 in leukemia development

POST DOCTORAL POSTER LIST / LISTE DES PROJETS EXPOSÉS DES POSTDOCTORAUX

P21	Aubert, Léo (2016-18)	Define the surfaceome landscape of hematopoieitic stem cells and pediatric leukemia specimens to improve the development of novel therapies
P22	Boulay, Karine (2017-19)	Targeted clearance of senescent cells towards improving cancer therapy
P23	Davis, Margaret (2016-18)	Novel functions of nuclear eukaryotic translation initiation factor (eIF4E) in leukemia
P24	Dubuissez, Marion (2016-18)	Role of Ikaros transcription factor in the transcription termination

P25	Khater, Fida (2016-18)	Tackling refractory/relapsed childhood cancer, one patient at a time
P26	Killoran, Ryan (2017-19)	Characterization of Dimer-Activated MLL and Probing the small GTPase Signaling Landscape in MLL-rearranged Cells
P27	Lavallée, Vincent-Philippe (2016-18)	Transcriptomic landscape of acute promyelocytic leukemia reveals aberrant surface expression of the platelet aggregation agonist Podoplanin
P28	Lebert-Ghali, Charles-Étienne (2016-18)	Modulation of T cell function by CD271
P29	Malina, Abba (2017-19)	Targeted screening for novel genetic driver mutations that elicit resistance to chemotherapy in Burkitts lymphoma using a novel CRISPR-based site-specific mutagenic approach
P30	Neault, Mathieu (2017-19)	Generation of a Mouse Model of Acute Megakaryoblastic Leukemia
P31	Olofsson, Linnéa (2017-19)	Whole genome CRISPR/Cas9 knock-out chemogenomic screens in a B-cell lymphoma cell line reveal specific drug mechanism-of-action and synergistic interactions
P32	Richard-Carpentier, Guillaume (2017-19)	High expression of SPAG1 is associated with a poorer clinical outcome in intermediate risk acute myeloid leukemia that can be partially overcome by hematopoietic stem cell transplantation
P33	Sood, Aditi (2016-18)	Optimizing the T cell repertoire for adoptive immunotherapy
P34	Steinberger, Jutta (2017-19)	Tracing Myc Expression for Small Molecule Discovery
P35	Tomelinni, Elis (2017-19)	Integrin-α3 as a new marker for cord blood derived hematopoietic stem cells: redefining the hierarchy of cultured stem cells
P36	Wever, Claudia (2016-18)	BH3 profiling reveals dependency on multiple anti-apoptotic proteins in primary non-Hodgkin lymphoma samples

P01

Title: Characterization of the functional impact of polymorphisms associated with asparaginase complications identified by exome-wide association study

Author: Rachid Abaji

Affiliation: Vincent Gagné, Romain Gioia, Christian Beauséjour, and Maja Krajinovic; Department of Pharmacology at the University of Montreal and Research Centre of CHU Sainte-Justine.

Key words: Acute lymphoblastic leukemia; asparaginase; pancreatitis; pharmacogenetics; CRISPR-Cas9.

Background information: We previously reported the results of an exome-wide association study using sequencing data which identified a list of SNPs associated with adverse drug reactions related to the administration of asparaginase (ASNase) during acute lymphoblastic leukemia treatment (ALL). Of those, rs3809849 in the MYBBP1A gene & rs11556218 in IL16 gene were associated with multiple complications and their association with pancreatitis was replicated in an independent validation cohort.

Purpose of the study: Our aim is to understand how these genetic alterations translate into differences in treatment response through cell-based functional analysis.

Methods: We performed a screening in lymphoblastoid cell lines (LCLs) to assess the impact of the risk alleles on ASNase sensitivity reflected by the change in IC50. We also produced knockouts of these genes in NALM6 (lymphoblastic) and PANC1 (pancreatic) cancer cell-lines using CRISPR-CAS9 technology and tested the difference in viability between the knockouts & wild-type (WT) cells following ASNase treatment.

Results: The screening demonstrated that both SNPs incurred a significant modulation of drug sensitivity in LCLs. Interestingly, the variant allele in IL16 rendered the cells more sensitive to ASNase while that of MYBBP1A was associated with more resistance. Our knockout ΔMYBBP1A-NALM6 cells showed a significant increase in resistance to a range of high doses of ASNase compared to WT cell. Both ΔMYBBP1A & ΔIL16 PANC1 cells were associated with resistance to ASNase reflected by a significantly higher IC50 48h after drug challenge. Interestingly, ΔIL16 cells maintained the increase in resistance even after 4 days of incubation, unlike ΔMYBBP1A which had no significant difference from WT cells at day 4. Furthermore, both knockouts showed differences in the morphology but ΔIL16 cells seem to reflect a more malignant and drug resistant phenotype.

Conclusions: Our results suggest a functional role of the two genes in modulating the sensitivity of the different cell-lines as well as the morphology of PANC1 cells. This further advocates the implication of the identified SNPs in influencing the outcome of acute lymphoblastic leukemia treatment with ASNase.

P02

Title: The Role of mutated Ras-associating proteins in relapse/refractory GCB-DLBCL

Author: Alexandre Benoit

Affiliation: Koren K. Mann. Lady Davis Institute, Jewish General Hospital; Montreal, Quebec. Division of Experimental Medicine, McGill University; Montreal, Quebec, Canada.

Keywords: DLBCL, R-CHOP resistant, mutations, Ras-associating proteins

Background information: Diffuse large B cell lymphoma (DLBCL) is a heterogenous disease, highlighted by the number of gene expression and mutational signatures identified. However, all patients currently receive the same treatment, the R-CHOP regimen, and despite significant efficacy, DLBCL relapses in approximately 40% of the patients. Although several groups have defined recurrent mutations in newly diagnosed DLBCL patients, we have focused on mutations found in R-CHOP resistant patients. We performed exome sequencing on 38 relapsed/refractory DLBCL (rrDLBCL) samples, where we utilized germline DNA from peripheral blood to define lymphoma-specific mutations. We have identified several Ras-associated proteins mutated in the biopsies taken at relapse including proteins playing a role in proliferation, survival, apoptosis, adhesion and migration. At relapse, 45% of the GCB patients and 21% of the ABC patients had a mutation in a Ras-associating protein. Interestingly, there were no mutations in RAS at relapse in our cohort. Additionally, at relapse there was a higher mRNA expression level of Ras and Ras-associating proteins in GCB-DLBCL samples compared to ABC-DLBCL samples.

Purpose of the study: Determine whether mutations in Ras-associating proteins lead to hyperactivation of the MEK/ERK survival pathway and mediate resistance to R-CHOP in GCB-DLBCL.

Methods and Results: By IHC we found that 31% of the relapse samples were positive for p-ERK compared to 14% in the diagnostic samples. Importantly, in paired biopsies, we observed that 29% gained p-ERK staining at relapse. Ras Guanyl Releasing Protein 4 (RasGRP4) was the most commonly mutated Ras-associated protein gene, and was mutated only in GCB-DLBCL subtype patients. RasGRP4 is a guanine nucleotide exchange factor (GEF) that activates Ras. When we simulated the stability of RasGRP4-hRas heterodimers by molecular dynamics, the presence of D404N or R280K point mutations stabilized the interaction between HRas and RasGRP4. In addition, we created human GCB-DLBCL cell lines with wild-type or patient-associated mutant RasGRP4. Mutant RasGRP4 cell lines had higher basal levels of phosphorylated ERK in normal serum condition, as well as hyperactivation of ERK after stimulation with PMA, but not anti-IgM. Although we expected a growth advantage correlating with the increased phosphorylation of ERK, the lymphoma cells expressing mutant RasGRP4 did not grow faster than the wild-type. We assessed the cytotoxic activity of component of R-CHOP and MEK inhibitors in the mutant and wild-type lymphoma cell lines by MTT assay in bulk culture. Here, RasGRP4 wild-type and mutant containing cells had a similar response to chemotherapies found in the R-CHOP regimen. However, in a single cell clonogenic assay the mutant RasGRP4 cells had a survival advantage compared to wild-type cells in the presence of doxorubicin.

Conclusion: Relapse in DLBCL after the standard of treatment is still considerable and the mechanism behind R-CHOP resistance is poorly understood. We believe these results provide a better understanding of R-CHOP resistance and will lead to suitable alternative therapies to treat DLBCL patients.

P03 (absent)

Title: CRISPR-based genome-wide knockouts to identify synthetic lethality with telomerase inhibition in acute lymphoblastic leukemia

Author: Yahya Benslimane

Affiliation: Thierry Bertomeu, Jasmin Coulombe-Huntington, 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 and leukemic cells re-activate telomerase (TERT) to counteract this shortening and become immortal. Telomerase is a very attractive target for inhibition due to its potentially broad therapeutic inhibition and the lack of expression of telomerase in somatic tissues. 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 B-ALL cells use specific processes to cope with telomere erosion and that these processes can be combined with telomerase inhibition to trigger synthetic lethality.

Methods: The CRISPR/Cas9 system has been used to precisely knockout genes and has been adapted to systematic genome-wide screening. A pooled library of sgRNAs with whole-genome targeting capability was introduced in the human NALM-6 cell line (pre-B ALL) and the cells were treated with BIBR1532, a potent telomerase inhibitor to induce telomere shortening.

Results: Illumina sequencing of the library was carried out followed by bioinformatic analysis of the sgRNA frequency distributions in cells treated with 20 μM for 20 days. Several genes showed a defect in fitness upon knockout specifically in the presence of the telomerase inhibitor, suggesting a chemo-genetic interaction. Using a genetic knockout of TERT in NALM-6 cells, further characterization of the hits was performed to eliminate interactions due to off-target effects of BIBR1532 and hone in on those that are dependent on the inhibition of telomerase activity.

Conclusion: This work has identified several candidates in the gene networks that can be targeted in combination with telomerase inhibition in order to hinder the mechanisms used by cells to cope with short telomeres. This will inform on novel alternative therapies that directly target telomerase in B-ALL. The design of these new combinatorial strategies is of special interest to acute leukemia patients who relapsed due to resistance to the currently available therapies.

P04

Title: Leukemic stem cell expression signatures identify novel therapeutics for acute myeloid leukemia

Author: Meaghan Boileau

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

Keywords: acute myeloid leukemia; leukemic stem cells; gene expression signatures; drug screen

Background information: Acute myeloid leukemia (AML) has a poor 5-year survival rate of 60% in pediatric patients and accounts for approximately half of leukemic deaths in children. This is largely due to high rates of relapse, thought to be driven by chemoresistant leukemic stem cells (LSCs). Unlike typical cancerous cells, LSCs are often quiescent, allowing them to evade standard therapies and serve as a reservoir for relapse. Thus, the identification of anti-LSC therapies could improve the clinical outcomes of patients suffering from AML.

Purpose of study: The aim of this study is to identify compounds that specifically target LSCs while sparing normal hematopoietic stem cells (HSCs) using LSC and HSC specific gene expression signatures.

Methods: To identify compounds predicted to impede LSC function while not affecting HSCs, we probed datasets of drug-gene interaction with our defined LSC and HSC gene expression signatures (Eppert et al. Nat Med, 2011), and an additional LSC-signature from 86 AML patients (unpublished data; Ng et al. Nature, 2016). We screened the identified compounds against a primary AML sample (8227) with a known LSC-containing population and assessed viability and immunophenotype by flow cytometry. We further tested candidate compounds against normal HSCs and additional primary AML samples, as well as functional leukemic progenitor cells in AML 8227 cells.

Results: From the in silico analysis, we identified 151 compounds predicted to target LSCs without harming HSCs. We screened 83 of these compounds against AML 8227. We identified multiple hits in two classes of drugs: 3 corticosteroids and 3 cardiac glycosides. In AML 8227, the corticosteroids differentiated primitive cells into terminally differentiated blasts while the cardiac glycosides preferentially eliminated the LSC and progenitor containing populations. Both classes had limited toxicity against HSCs. We assessed the compounds in multiple primary AML samples and observed heterogenous response, likely due to the genetic drivers present in each sample. Steroids differentiated AML 9642 but did not affect AML 9706 and cardiac glycosides were effective against AML 184 while AMLs 9642, 9706 and 116 were more resistant. Treatment of AML 8227 with the steroids or cardiac glycosides resulted in a 2-fold decrease in functional progenitors compared to DMSO.

Conclusion: We establish that the corticosteroids and cardiac glycosides identified by our bioinformatic approach have anti-AML and anti-LSC properties against AML samples in vitro. Next steps include examining response in a panel of AML samples to elucidate a link between clinical or genotypic features and responsiveness. We will also perform xenotransplantation of treated AML samples into immunodeficient mice to assess the effects on functional LSCs. Results from our study will provide valuable insight to LSC biology and can lead to new therapeutic approaches for targeting the LSCs, the root of therapy resistance and relapse in AML.

P05

Title: Atypical visual short-term memory activation patterns in acute lymphoblastic leukemia survivors: A MEG study

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Keywords: visual short-term memory, neural correlates, 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 deficits in short-term memory. Despite a good knowledge of the cognitive deficits in ALL survivors, few studies have looked at the neural correlates associated with the impaired cognitive functions, like short-term memory, in that population.

Purpose of the study: The main objective of the study was to investigate abnormalities in performance and neural correlates of visual short-term memory (VSTM) in a cohort of long-term ALL survivors.

Methods: A total of 41 ALL survivors and 26 controls completed a VSTM task in magnetoencephalography. To investigate performance on the task, we computed Cowan's K (Cowan, 2001), which is an index of VSTM capacity. MEG analyses at the source level, using MNE was used to investigate brain activation during the retention period of the VSTM task (400-1400ms). Monte-Carlo Permutation test (2000 randomizations) was used to assess differences between groups.

Results: There was no difference in performance (Cowan's K) between ALL survivors and controls during the VSTM task. However, ALL survivors recruited additional cortical areas when performing the task, including lateral occipital cortex, precentral and postcentral gyrus as well as superior temporal and middle temporal gyrus ($p<0.05$).

Conclusion: Our results showed that survivors exhibited more widespread activation than the controls during the VSTM task. In addition to recruiting regions that are usually involved in VSTM, they also recruited regions that are not typically known to play a role in such tasks. Our results suggest the existence of compensatory neural mechanisms in ALL survivors, allowing them to perform equally as healthy subjects.

P06

Title: Cancer-related effects on relationships, long-term psychological status and relationship satisfaction in couples whose child was treated for leukemia: A PETALE study

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Keywords: pediatric leukemia, couples, interdependence, impact of cancer, adjustment

Background information: According to follow-up studies, the psychosocial impact of pediatric cancer on parents often extends beyond the end of their child's cancer treatments. In fact, parents can continue to experience both individual and relationship effects up to several years following treatment completion.

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

Methods: Parents of childhood ALL survivors (n = 103 couples) were surveyed as part of a cohort recall (PETALE cohort). Both parents completed questionnaires exploring adjustment (Brief Symptom Inventory-18, Dyadic Adjustment Scale) and perceived impact of cancer on the relationship (Impact of Cancer on the Couple). Mothers' and fathers' scores were compared using MANOVAs. We also examined the degree to which a parent's perceived changes in relationship dynamics following their child's cancer were associated with their own current adjustment (actor effects), and their partner's current adjustment (partner effects) using the Actor-Partner Interdependence Model (APIM).

Results: Frequencies of current distress were normative in parents (mothers/fathers): general distress (6.8/7.8%), anxiety (5.8/6.8%), depression (2.9/6.8%), somatization (13.6/9.7%), and relationship distress (21.4/20.4%). Mothers and fathers typically agreed on their reported relationship satisfaction, and the perceived nature of relationship changes following the illness. Dyadic analyses indicated that whereas mothers' adjustment was related to their own perceived relationship changes, fathers' adjustment was primarily related to their partner's perceptions.

Conclusion: In long-term stable couples, mothers may act as an influential bridge connecting the illness experiences of survivors and fathers. This could explain why mothers' perceptions of relationship changes were related to their partners' long-term adjustment, which was not the case for fathers.

P07

Title: Oncogenic context determines therapeutic and epigenetic efficacy of cardiac glycosides in paediatric leukemia

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Key words: Acute lymphoblastic leukemia, C-MYC oncogene, histone acetyltransferase, drug repurposing.

Background information: In a drug screening initiative, we recently reported that proscillaridin, a FDA-approved cardiac glycoside, exhibits unsuspected epigenetic and anticancer activities. With the aim of repurposing proscillaridin for cancer treatment, we treated a panel of 14 cancer cell lines with proscillaridin and found that leukemia cells were the most sensitive to the treatment.

Purpose of the study: To discover proscillaridin epigenetic and anticancer mechanism of action in acute lymphoblastic leukemia.

Methods and Results: To gain insight into high sensitivity of leukemia cells to proscillaridin, we compared the expression of C-MYC oncogene, involved in stimulating the hematopoietic transformation and commonly deregulated in leukemia, in the panel of 14 cancer cell lines. Leukemia cell lines exhibited the highest expression of C-MYC and we measured a negative correlation between C-MYC expression and proscillaridin IC50. Proscillaridin (5 nM; 48h) induces 50% downregulation of C-MYC protein level in leukemia cell lines expressing high C-MYC level. Conversely, proscillaridin did not affect C-MYC protein level in low C-MYC expressing cell lines. To demonstrate the driver role of the oncogenic context in proscillaridin sensibility, we transfected human primary fibroblasts either with C-MYC oncogene or with RAS oncogene. Proscillaridin anticancer activity was 70 times higher in fibroblasts transfected with C-MYC than fibroblasts transfected with RAS. In a human T acute lymphoblastic leukemia cell line expressing a high level of C-MYC, proscillaridin treatment (5 nM; 48h) resulted in a transcriptomic shift of proliferative programs to T-cell differentiation and activation. Proscillaridin produced global chromatin remodelling by removing 75% of H3 acetylation, which resulted in the downregulation of genes involved in cancer cell proliferation and most importantly, of C-MYC target genes. Mechanistic studies by Western blot and mass spectrometry revealed that proscillaridin produced an epigenetic reprogramming by decreasing the expression of histone acetyltransferases, resulting in a decrease in acetylation of C-MYC, C-MYC targets and chromatin regulators.

Conclusions: For the first time, we demonstrate that C-MYC brings a specific oncogenic context in which KAT inhibition by proscillaridin will play a driver role in remodelling chromatin to inhibit proliferative programs and induce leukemia cell differentiation. We propose that proscillaridin can be repurposed as a new epigenetic drug in high C-MYC expressing cancers such as acute lymphoblastic leukemia.

Conclusion: TET2 is a critical tumor suppressor highly mutated in hematological malignancies and our study shed more light on how TET2 is regulated on both mRNA levels and protein levels in AML cells and other cancer types. Also we identified how TET2 catalytic activity towards 5mC is regulated by TET2's major partners OGT. TET2 is a member of TET family which lacks a DNA binding domain and our study has identified CXXC1 as a new linker between DNA and TET2.

P08

Title: G Protein-Coupled Receptors 56 and 114 as Potential Functional Regulators of Normal and Leukemic Human Stem Cells

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Keywords: Acute myeloid leukemia; G protein-coupled receptors; 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 is a G-protein coupled receptor (GPCR) 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. GPR114 is a paralog of GPR56 that may also play a functional role in the regulation of HSCs and LSCs.

Purpose of the study: This study aims to establish the role of GPR56 and GPR114 in primary human LSCs and HSCs. Insight into the function of GPR56 and GPR114 as human stem cell regulators will reveal their potential as targets for improved therapy directed towards LSCs. Methods: Expression levels were determined in flow-sorted samples by qRT-PCR. Microarray expression data was obtained from 3 cohorts, one including cytogenetically abnormal AML. Cells were transduced with lentivirus to overexpress the GPCRs. Progenitor activity was assessed by colony forming cell assay by plating flow-sorted samples in methylcellulose with human cytokines for 12-14 days. Lineage negative human cord blood cells were transduced then intrafemorally injected into SCID immune-deficient mice. Xenograft efficiency was assessed after 12 weeks by flow cytometry. **Results:** Expression of GPR56 and GPR114 was higher in LSCs, normal HSCs, and normal progenitors versus mature populations in normal or leukemic blood. Above-median expression of GPR56 or GPR114 was correlated with shorter survival ($p<0.01$). Expression of both GPR56 and GPR114 were higher samples with worse outcome predicted by cytogenetics ($p<0.0001$) and lower in with better outcome predicted by cytogenetics (GPR56: $p<0.01$, GPR114: $p<0.05$). Overexpression GPR56 and GPR114 increased leukemic progenitor activity in MOLM13 AML cell line (GPR56: $p<0.05$, GPR114: $p<0.01$) and cultured AML sample 8227 (GPR56dN $p<0.0001$, GPR114: $p<0.01$); however, hematopoietic progenitor activity was unaltered. In xenograft assay for HSC function, GPR56 overexpression conferred a significant engraftment advantage versus hRluc control ($p<0.0001$). This advantage was maintained in secondary 12-week transplants ($p<0.05$) with unaltered percentage of stem and progenitor cells, indicating self-renewal of stem cells.

Conclusions: These data suggest that GPR56 and GPR114 may regulate human leukemic progenitors, but not hematopoietic progenitor cells, and that GPR56 enhances HSC function in vivo. Further functional studies will be performed to determine the role of GPR114 in HSCs, the roles of both GPCRs in LSC function in vivo, and their roles in chemotherapy resistance

P09

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

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Keywords: T-cell Acute Lymphoblastic Leukemia, pre-Leukemic Stem Cells, survival, mTOR pathway.

Background information: Ectopic expression of the SCL oncogenic transcription factor and its nuclear partner LMO1 or LMO2 activates a self-renewal gene network that confers sustained self-renewing properties to non-self-renewing DN3 thymocytes. In addition, pre-Leukemic Stem Cells (pre-LSCs) are absolutely dependent on the NOTCH1-MYC pathway (Gerby et al, PLoS Genet, 2014). Pre-LSCs have been associated with disease onset, maintenance and relapse, underscoring the importance of targeting pre-LSCs for more selective and better tolerated treatment regimens in childhood leukemias. Through an unbiased multiparameter screen with known pharmacological inhibitors, we have identified 2-ME2 as an inhibitor of pre-LSC viability and self-renewal activity via its capacity to prevent S6 phosphorylation and to inhibit MYC translation (Gerby et al, J Clin Invest 2016). Moreover, all inhibitors of the mTOR pathway that were present in the screened library scored as hits. Together, the results concur with the primordial importance of the mTOR pathway for pre-LSCs.

Purpose of the study: To define [1] the role of the mTOR pathway on pre-LSC self-renewal and survival and [2] the pathways through which 2-ME2 inhibits pre-LSCs.

Methods and Results: 2-ME2 is reported to be a microtubule targeting drug. However, we previously showed that pre-LSCs that escaped treatment are slowly dividing cells, raising the possibility that 2-ME2 inhibits pre-LSC viability through other mechanisms. Indeed, a counter screen with quiescent normal hematopoietic stem cells clearly showed that these cells are resistant to 2-ME2 compared to pre-LSCs. In the past year, we have demonstrated the dependence of pre-LSC cell survival on the mTOR pathway by establishing dose-response curves with known PI3K/mTOR inhibitors (Sirolimus, PI-103, PP242) acting at different levels of the pathway. Moreover, we showed all human ALL cells tested to be sensitive to 2-ME2. We next demonstrate by proteomic analysis that 2-ME2 inhibits translation and ribosome biogenesis, two processes controlled by the mTOR pathway, consistent with the essentiality of MYC targets and MTORC1 signaling revealed by a recent CRISPR screen in human lymphoblastoid cells (Berthomeu et al, Mol Cell Biol, 2017).

Conclusions: In summary, our results indicate that drug-resistant pre-LSCs as well as fully transformed leukemic cells are highly vulnerable to inhibition of the mTOR pathway and highlight the possibility of inhibiting this pathway in acute lymphoid leukemias while sparing normal hematopoietic stem cells.

P10 (absent)

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

Author: Martin Karam

Affiliation: Alexandre Orthwein, Experimental Medicine, McGill University

Keywords: DNA double-strand break, Homologous recombination, Fanconi Anemia, Acute Myeloid Leukemia

Background information: Fanconi Anemia (FA) is a hereditary disease characterized by bone marrow failure and predisposition to acute myeloid leukemia (AML). Most FA patients develop AML during childhood due to problems during hematopoiesis. Up to now, twenty FA genes have been characterized and linked to FA predisposition. All of them play a critical role in the response to DNA damage including DNA double-strand break (DSBs). This type of DNA lesion is particularly cytotoxic, as one single DSB can lead to cell death or result in major genomic rearrangements that favor carcinogenesis. More recently, an additional FA gene has been identified, REV7. We and others have shown that REV7 plays a critical role to the DSB response. Additional factors are involved in this pathway to promote DSB repair and may be linked to FA anemia and AML predisposition. We recently identified POGZ, by a mass spectrometry approach, and like REV7, POGZ knock-down results in impaired DSB response, raising the possibility that POGZ may also be a FA gene with great implication for pediatric AML development.

Purpose of the study: The goal of my project is to decipher the role of POGZ during hematopoiesis and pediatric AML development. I hypothesize that POGZ promotes proper hematopoiesis by stimulating optimal DSB repair in hematopoietic stem cells and prevents leukemogenesis.

Methods: Multiple reporter assays were used to demonstrate the role of POGZ in the DSB repair pathway. Reporter assays such as the DR-GFP, EJ5-GFP, and SA-GFP assays to show a role for POGZ in homologous recombination, non-homologous end-joining, and single strand annealing respectively were utilized. Then experiments were performed to determine if depleting POGZ would affect the cell cycle distribution as well as the sensitization of cells to DNA damaging agents. Finally, experiments to determine if POGZ is recruited to the site of damage were also performed.

Results: I started by deciphering the role of POGZ in the regulation of DSB repair, and using the multiple approaches mentioned above, I was able to conclude that POGZ indeed plays a crucial role in the DSB repair pathway whereby depleting POGZ resulted in HR, NHEJ and SSA impaired repair. Moreover, depleting POGZ did not affect the cell cycle distribution, yet it hypersensitized cells to DNA damaging agents such as PARP inhibitors. Finally, using multiple immunofluorescence approaches, we were able to show that POGZ is not recruited to the sites of damage.

Conclusion: My data shows that POGZ plays a critical role in the DNA damage response, and it holds a great potential for further understanding the FA pathway as well as the development of leukemia. Next, the aim is to validate these findings in HSCs and AML. Importantly, the culmination of these efforts will unveil novel biomarkers for the diagnosis of FA syndrome and pediatric AML. It may also allow to identify POGZ as a novel therapeutic target for pediatric AML treatment.

P11

Title: Modulation of co-signaling to improve ex vivo human antigen-specific T cells generation for immunotherapy

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Keywords: Adoptive Cell Therapy, TIM3, PD-L1

Background information: T cell-based immunotherapies are a promising approach to treat cancers and infections. However, various obstacles limit effective T-cell generation ex vivo, including terminal T-cell effector differentiation, exhaustion and eventually cell loss. The acquisition of dysfunctional features and terminal differentiation predicts for lack of proliferation and persistence following adoptive transfer, leading to poor efficacy. Co-inhibitory or "immune checkpoint" receptors play important role in T cell differentiation and fate. Our data showed that following ex vivo human T-cell stimulation with peptide loaded dendritic cells, a high fraction express the co-inhibitory receptors PD-1 and TIM3, along with PD-L1 (the main PD-1 ligand) (26% of total T-cell population). In addition, PD-L1 is also highly expressed on the peptide loaded dendritic cells (about 98% of total mature dendritic cell population) that are used to stimulate the T cells, thereby offering an explanation for the acquisition of dysfunctional features in ex vivo expanded antigen-specific T cells upon repeated antigen exposure.

Purpose of the study: To determine whether co-signaling modulation in antigen-specific T-cell cultures during ex vivo expansion will enhance T-cell yield and function.

Methods: First, using repeated stimulations of human T cells with peptide-pulsed antigen presenting cells, we generated antigen-specific T cells that gradually acquire exhaustion features then, ex vivo expanded antigen specific T cells are being treated with TIM3 and/or PD-L1 inhibitors and T cell activation, proliferation and cytokine production have been assessed following the treatment.

Results: Antigen specific T cells express high inhibitory receptors PD-1/PD-L1 and TIM3 (more than 50% of cells are double positive for PD-1 and TIM3), along with high levels of CD57 and KLRG1. PD-L1 and TIM3 blocking antibodies were introduced to co-culture at different time-points and different concentrations to generate antigen specific T cells without inducing exhaustion. The combination of anti-PD-L1 and anti-TIM3 increased total cell proliferation (two-fold increase in combination of PD-L1 and TIM3 blockade) and also yielded higher Ag-specific T cells with lower terminal differentiation and exhaustion markers. TIM3 + PD-L1 treated cells displayed a high proportion of central memory (Tcm) T cells and a low proportion of the more differentiated effector memory T cells (Tem) relative to single blockade and control conditions (no inhibitor used).

Conclusion: Together, these results suggest that PD-L1/TIM3 inhibition can improve ex vivo antigen-specific T-cell yield and confers a favorable phenotypic and functional features for improved immunotherapy. As such, co-signaling modulation during ex vivo T-cell cultures should be explored in current T-cell manufacturing protocols.

P12

Title: Transcriptomic landscape of acute promyelocytic leukemia reveals aberrant surface expression of the platelet aggregation agonist Podoplanin

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

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

Purpose of the study: Despite their clinical relevance, identifying TSAs remains a challenge since they derive from patient-specific cancer-induced genomic alterations.

Methods: As people only study TSAs derived from coding regions, we developed a novel proteogenomic workflow able to characterize the full TSA landscape of any given tumor, i.e. including TSAs derived from non-coding regions, which represent 98% of our genome. Briefly, using RNA-sequencing data, we subtracted the mTEChi signal from the tumor signal to generate tumor-specific protein databases enriched in non-tolerogenic sequences that can then be used to analyze the MAP repertoire of any sample sequenced by mass spectrometry.

Results: As a proof-of-concept, we first analyzed the MAP repertoire of two murine cell lines (CT26, a Balb/c colon carcinoma, and EL4, a C57BL/6 T lymphoma). We identified a total of 21 TSAs, 90% of which derived from allegedly non-coding regions. Interestingly, our results highlighted that 70% of those TSAs derived from non-mutated yet tumor-restricted sequences, e.g. endogenous retroelements. Focusing on 5 TSAs, we demonstrated that both TSA expression and TSA-specific T-cell frequency in the pre-immune repertoire influenced the overall survival of pre-immunized tumor-bearing mice. Finally, using this very same proteogenomic workflow to analyze 7 human primary tumor samples (3 lung tumor biopsies and 4 B-ALL specimens), we confirmed that, just like murine TSAs, most human TSAs derive from non-coding and non-mutated sequences.

Conclusion: Here, we demonstrate that, in both mouse and human, non-coding regions are the main source of TSAs. Since most of those TSAs derive from non-mutated sequences yet tumor-specific sequences, their discovery should help expand the TSA landscape of lowly mutated cancer types, e.g. leukemia, while providing targets that could be shared by multiple patients. Altogether, our study provides a solid framework for identifying and selecting the best antigens for T-cell based cancer immunotherapies.

P13

Title: OTUB1 in B cell development

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Keywords: B cell, splenic marginal zone lymphoma, Marginal Zone, Spleen

Background information: The mouse spleen is a crucial organ that functions in immune surveillance. In particular, marginal zone B cells are strategically positioned in between the white pulp and red pulp of the spleen. This facilitates interactions of MZ B cells with circulating antigens as a result of the low flow rate of the blood passing through the Marginal zone. Yet, the development of the marginal zone subtype is not well understood.

Ubiquitination is a post-translational modification that plays an essential role in several biological processes, including key aspects of the immune response as well as the signaling of the cytotoxic DNA double-strand break (DSB) lesions. Like phosphorylation, the attachment of ubiquitin (Ub) to targeted proteins is a reversible process, catalyzed by specific deubiquitinating enzymes (DUBs). The DUBs Ovarian Tumor Domain containing Ubiquitin Aldehyde Binding protein 1 (Otub1) and 2 (Otub2) were shown to be central in tuning the ubiquitination cascade that signals and modulates the repair of DSBs.

Purpose of the study: To understand the role of deubiquitinase OTUB1 in the development of marginal zone B cells. In addition, we are interested to understand how OTUB1 might affect the development of splenic marginal zone lymphoma.

Results: Here, we show that Otub1, unlike Otub2, is essential for proper B-cell maturation. While loss of Otub1 in the B-cell compartment has limited impact in the bone marrow, its deletion results in a drastic accumulation of marginal zone (MZ) B-cells in the spleen (~4-fold increase), which is dependent of its catalytic activity. This phenotype correlates with a gut dysbiosis in Otub1-targeted mice, where the Alistipes genus is more abundant. However, it remains unclear how Otub1 loss results in a follicular to marginal zone shift and how it impairs gut microbiota in mice. To answer this question, we used a systematic proximity-based biotin labelling approach coupled to mass spectrometry, called BioID in order to decipher the interactome of Otub1. There, we identified the lymphoid-specific helicase HELLS and the DNA methyltransferase DNMT1 as novel Otub1 proximal/interacting factors and we are currently validating their relevance in regard to Otub1.

Conclusion: Altogether, this work identifies a novel function of the DUB Otub1 in the immune response, where it regulates proper B-cell maturation in secondary lymphoid organs, independently of its key role in genome integrity.

P14

Title: X-linked dyskeratosis congenita mutations disrupt the dyskerin-telomerase RNA interaction

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

Background: Defective maintenance of DNA ends (telomeres) is the cause of the premature aging syndrome dyskeratosis congenita (DC). Patients with DC experience early onset of age-related maladies, including a risk of blood malignancies such as acute myeloid leukemia and non-Hodgkin lymphoma. DC is classified as a bone marrow failure syndrome, with BMF accounting for the main cause or mortality in patients. The H/ACA ribonucleoprotein component dyskerin is implicated in the most common form of heritable DC, X-linked DC (X-DC). Dyskerin is integral to telomere maintenance through regulation of telomerase assembly and stability. Our lab previously established that impairing dyskerin SUMOylation with X-DC mutations in the N-terminal X-DC hotspot negatively affects telomerase RNA (hTR) levels and telomere maintenance.

Purpose: The objective of this project is to understand how X-DC mutations affect dyskerin function, and ultimately telomere maintenance. We are characterizing disease-causing substitutions in dyskerin that affect SUMOylation and telomerase RNA biogenesis.

Methods: SUMOylation sites that are mutated in X-DC were identified by consensus prediction and mass spectrometry confirmation. These identified sites fall outside of the functional domains of dyskerin and have not been previously characterized for dyskerin function. Dyskerin variants defective for SUMOylation at disease-implicated lysine residues were examined for RNA interactions by co-immunoprecipitation and qPCR; localization by immunofluorescence; protein complex assembly by co-immunoprecipitation; and RNA stability and processing by dyskerin knockdown paired with qPCR. DC-causing variants of dyskerin previously reported to display RNA-binding defects were also examined.

Results: All X-DC variants examined to date are able to form H/ACA ribonucleoprotein complexes and localize to sites of maturity. Some X-DC variants have impaired RNA interactions specific to hTR, a potential contributing cause of X-DC in patients harboring amino acid substitutions at these sites through destabilization of hTR. This specificity is in contrast to the disruption of various H/ACA RNAs including hTR that is observed upon a total loss of dyskerin (not observed in patients). This observation more closely resembles the interaction defects of mutations within the putative RNA binding domain of dyskerin. The hTR interaction defect observed for all of the X-DC variants in this study includes defective interaction with poly-adenylated precursors of hTR, as well as a loss of poly-adenylated hTR precursors and lack of extended hTR species upon dyskerin depletion.

Conclusion: Analyzing the molecular consequences of X-DC 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 interactions specific to hTR, leading to hTR degradation and telomere maintenance defects. The hTR interaction defect observed between the X-DC variants and poly-adenylated precursors of hTR, combined with the observed

lack of poly-adenylated hTR precursors and extended hTR species upon dyskerin depletion, indicate that X-DC mutations that disrupt the interaction between dyskerin and hTR likely affect the levels of hTR by favouring degradation over correct processing, as has been a suggested role of dyskerin in hTR biogenesis.

P15

Title: Dyslipidemia in pediatric acute lymphoblastic leukemia survivors: a role for gut microbiota

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Keywords: Dyslipidemia, gut microbiota, acute lymphoblastic leukemia survivors, xenogeneic murine model.

Background information: Although available literature reports metabolic disturbances in childhood acute lymphoblastic leukemia (cALL) survivors, the precise etiology and underlying mechanisms of these long-term complications are poorly understood. Evidence supports the implication of changes in the gut microbiota in the development of oxidative stress and peripheral inflammation that may lead to dyslipidemia, obesity and insulin resistance. However, no studies to our knowledge have evaluated the impact of changes in gut microbiota caused by cancer, treatment, and diet in the development of the metabolic complications observed in cALL survivors.

Purpose of the study: This study aims to: 1) examine the potential alterations in lipid profile and lipoprotein composition in children and young adults survivors of cALL; 2) study, in this group, the correlation between gut microbiota composition and cardiometabolic complications and; 3) investigate, in a mouse model of cALL, the mechanisms causing these metabolic alterations.

Methods: 1) Lipid and lipoprotein profiles were analyzed for cALL survivors recruited as part of the PETALE study at SJUHC (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 analyzed by enzymatic tests and SDS-PAGE, respectively. 2) A faecal sample was collected in a sub-cohort of 37 cALL surivors with zero and extreme cardiometabolic phenotypes. The composition and diversity of the gut microbiota will be compared between the 2 groups. 3) Immunodeficient mice will be grafted with human leukemic cells and will be treated with methotrexate, followed by thorough metabolic and gut microbiota characterization.

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. HDL2 had reduced Apo A-I/A-II ratio in cALL survivors compared to controls. Analysis of VLDL-Apo Cs disclosed a trend for higher Apo C-III1 content in dyslipidemic cALL survivors. For faecal analysis, we have identified 19 survivors with extreme phenotype and 18 age and gender-matched survivors without any cardiometabolic perturbation. Overweight and obesity affected 100% of survivors with extreme cardiometabolic phenotype. The prevalence of pre-hypertension and hypertension was 26% in this group. Insulin resistance and dyslipidemia were mainly present with 79% and 84%, respectively. So far, on 12 patients contacted, 67% of samples were received.

Conclusion: Understanding of the etiology of long-term complications will undoubtedly improve the monitoring, prognosis, prevention and treatment strategies of cALL survivors. Our work could contribute to the development of nutritional strategies aimed at restoring the intestinal flora and exploiting the benefits of nutritional therapy during cancer treatment in children.

P16

Title: Smarcd1 Subunit of SWI/SNF Complexes is Required for Normal and Pathogenic Lymphopoiesis

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Keywords: Epigenetics, SWI/SNF complexes, Smarcd1, Lymphopoiesis, Lymphoma

Background information: Lymphocyte development from hematopoietic stem cells (HSCs) is accompanied by a loss of self-renewal capacity and a progressive restriction of developmental potential. Although the molecular mechanisms for generation of mature B or T lymphocytes are beginning to be revealed, a major unanswered question is how the multipotent progeny of HSCs initiate commitment toward lymphoid fate. Dysregulation of these processes can lead to malignant transformation and development of lymphomas. Our recent studies revealed that Smarcd1, a subunit of SWI/SNF chromatin remodeling complexes, is essential for early lymphopoiesis and lymphoma progression.

Purpose of the study: This study aims to identify the molecular pathways that are regulated by Smarcd1 during normal and pathogenic lymphopoiesis.

Methods: 1. To study the role of Smarcd1 during lymphoid development, different mouse models allowing acute Smarcd1 deletion at different developmental checkpoints during B- and T- cell development have been generated.

2. To study the role of Smarcd1 in lymphoma, RNA interference experiments have been performed in human lymphoma cell lines.

Results: 1. Acute deletion of Smarcd1 in adult hemopoiesis using a Smarcd1fl/fl-Mx1Cre mouse model causes severe lymphopenia with near complete absence of mature B- and T-lymphocytes, whereas the myeloid and erythroid lineages remain unaffected. Common lymphoid progenitors (CLP) and lymphoid primed multipotent progenitors (LMPP) are severely reduced, indicating a role for Smarcd1 in early lymphoid commitment. Interestingly, Smarcd1 is required transiently in the lymphoid lineage since its inactivation in committed B- and T-cells (using CD19- and CD4-Cre transgenics, respectively) is compatible with lymphoid maturation. Transcriptomics analyses in multipotent progenitors revealed a functional collaboration between Smarcd1 and the E2A transcription factor in inducing the lymphoid gene program. Similar to E2A, Smarcd1 is also required for expression of the Rag1/2 and TdT recombinases that promote V(D)J recombination during lymphocyte maturation.

2. SMARCD1 depletion in lymphoma cell lines induces proliferation arrest at the G1/S phase of the cell cycle and increased cell death. These include high-grade B-lymphoma cell lines with MYC and BCL2 and/or BCL6 rearrangements, so-called “double-hit” lymphomas, which have poor prognosis and represent a major unmet medical need.

Conclusion: Altogether, these studies identified Smarcd1 as a lineage-specific chromatin remodeler that specifies cell fate, regulates cell growth and enforces developmental checkpoints in the lymphoid lineage. This work may eventually lead to a better management of diseases affecting lymphoid function and the development of strategies targeting new therapeutic targets.

P17

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, chromatin remodeling, bioinformatics

Background information: In acute myeloid leukemia (AML), malignant leukemic stem cells acquire an unlimited potential for self-renewal and are blocked in an undifferentiated state. AMLs are characterized by irreversible genetic rearrangements, such as the MLL-AF9 gene fusion, as well as the potentially reversible dysregulation of epigenetic mechanisms that modulate gene expression, genome stability, and nuclear architecture. We and colleagues have previously determined that the expression levels of KDM4A, a JUMONJI-family lysine demethylase, are elevated in tissue samples derived from pediatric MLL-AF9 AML patients. Through chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) analysis, we have identified the localization of KDM4A at many genomic loci that are enriched in epigenetic modifications characteristic of regions known as transcriptional enhancers. We hypothesize that KDM4A participates in leukemogenic gene expression programs by binding to genomic enhancers in pediatric MLL-AF9 AML.

Purpose of this study: We aim to characterize the precise epigenetic function of KDM4A in pediatric MLL-AF9 AML. Specifically, we will determine the genomic localization of KDM4A at novel genomic enhancer elements in AML and its role in modulating gene expression and chromatin architecture.

Methods: Using molecular inhibition methods followed by transcriptomics and ChIP-seq analyses, we are investigating gene programs that are modulated by KDM4A in human and mouse leukemic cells carrying the MLL-AF9 fusion. These experiments are followed by bioinformatics analyses to determine differences in gene expression and chromatin configuration. In addition, we are examining the effect of KDM4A depletion in the recruitment of other chromatin binding proteins known to participate in leukemic maintenance. To further characterize the phenotypes observed upon KDM4A depletion in MLL-AF9 leukemia, we are performing fluorescence assisted cell sorting assays including a variety of cell surface markers.

Results: The depletion of KDM4A leads to cellular differentiation and senescence in MLL-AF9 cell lines, suggesting that KDM4A plays a critical role in maintaining leukemic proliferation. Importantly, this phenotype is restricted to leukemic cells, while normal HSPCs are spared upon KDM4A knockdown. This phenotype is accompanied by the transcriptional modulation of several key genes involved in leukemogenesis. KDM4A depletion also results in the decreased chromatin binding of other epigenetic regulators known to promote oncogenic gene expression via genomic enhancers.

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 enhancer landscapes are epigenetically modulated by KDM4A in leukemic stem cells and in MLL-AF9 mouse models.

P18

Title: The master transcription factor RUNX1 antagonizes the Glucocorticoid Receptor pathway in Acute Myeloid Leukemia

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Keywords: Acute Myeloid Leukemia; RUNX1; chemogenomics; CRISPR-Cas9 screen; glucocorticoids.

Background: RUNX1 is an essential transcription factor for definite hematopoiesis and plays important roles in immune functions. Mutations found in RUNX1 occur in 5-13% of Acute Myeloid Leukemia (AML) patients (RUNX1mut) and characterize a specific subgroup associated with poor outcome. Recently we showed that RUNX1 allele dosage dictates sensitivity to glucocorticoids (GCs) in RUNX1mut AML patient cells, unravelling a new role for RUNX1 in the Glucocorticoid Receptor (GR) pathway. GR is a nuclear receptor that modulates the expression of thousands of genes involved in several biological processes such as metabolism, immune function, skeletal growth, etc. GCs are commonly used to treat cancers of the lymphoid system; however, their potential use in AML as well as the mechanism of action of these drugs are not fully understood.

Purpose of the study: This study aims to establish the genetic determinants of GCs-sensitivity and the molecular mechanism by which RUNX1 antagonizes this response in AML.

Methods and Results: RNA sequencing of RUNX1mut and RUNX1wild-type primary AML specimens from the Leucegene cohort was performed. Transcriptome analyses identified GR transcripts (NR3C1) as one of the transcripts whose expression is determined by RUNX1 allele dosage and showed increased expression in RUNX1mut specimens, indicating that RUNX1 inactivation could lead to GR upregulation and subgroup sensitivity to GCs. According to what was observed in primary specimens, acquired sensitization to GCs by RUNX1 knockdown in human AML cell lines was accompanied by upregulation of GR at transcript and protein levels. However, basal levels of GR couldn't explain GCs-sensitivity in all cases, indicating that other mechanisms involving RUNX1 and GR might collaborate to the GCs-response. Using co-immunoprecipitation (co-IP) experiments we demonstrated that RUNX1 and GR physically interact in AML cells. Overexpression of FLAG-tagged RUNX1 mutants in HEK293 cells followed by co-IP identified the C-terminal Inhibitory Domain of RUNX1 as essential for the interaction. Our results suggest that RUNX1 could be part of the GR transcriptional complex and modulate the transcription of genes involved the in response to treatment. In order to identify genes involved in this pathway, we will perform a genome-wide CRISPR-Cas9 screen in AML cells in the presence of GCs. For that, we generated RUNX1 normal (parental line, GCs-resistant) and RUNX1-deficient (GCs-sensitive) AML cell lines that uniformly express Cas9 under a doxycycline-inducible promoter. The genetic screen will be conducted in GCs-supplemented media for the identification of genes that confer sensitivity and resistance to GCs.

Conclusions: Our data shows that RUNX1 modulates GR levels and influences GCs-sensitivity in AML. We also observed that RUNX1 and GR physically interact in AML cells, and this association is linked to GC-resistance. By deciphering the GR pathway and its interaction with RUNX1, we will gain mechanistic insights that will facilitate the design of more effective therapeutic strategies for poor outcome AML patients.

P19

Title: A novel mechanism of regulation of the tumor suppressor BAP1/ASXL2 complex by monoubiquitination

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Keywords: H2A Ubiquitination, Cancer biology, BAP1/ASXL2, Monoubiquitination, tumor suppression.

Background information: Monoubiquitination of histone H2A K119 (H2Aub) is a critical epigenomic modification associated with development, cell proliferation and cancer. In Drosophila, H2A-ub is reversed by the deubiquitinase (DUB) and Polycomb group protein Calypso, which associates with Additional Sex Comb (ASX) and forms the Polycomb Repressive DUB (PR-DUB) complex. We previously reported that the tumor suppressor and mammalian ortholog of Calypso, BAP1, forms two mutually exclusive complexes with ASXL1 and ASXL2, two orthologs of ASX which are required for stimulating its catalytic activity and regulating BAP1 function in cell cycle control.

Purposes of the study: we found that BAP1/ASXL2 complex is regulated by monoubiquitinations. But, it remains functionally and mechanistically unclear how ASXL1 and ASXL2 are regulated by ubiquitination and how this impacts the deubiquitinase activity and function of BAP1.

Methods: we immunoprecipitated ASXL1 and ASXL2 following expression in 293Tcells with and without BAP1 and HA-ubiquitin. After mass spectrometry analysis for ubiquitination sites identification, we used ASXL2 domain deletion mutants and found that the ASXM2 domain is necessary for monoubiquitination. We evaluated the monoubiquitination effect on cell cycle progression and whether that monoubiquitination of ASXL2 could affect its stability by using proteasome inhibition with Mg132 and cycloheximide chasse. We conducted an E2-conjugating siRNA screen to further identify components that regulate ASXL monoubiquitination. To evaluate the eventual impact of ASXM2 monoubiquitination on BAP1 activity, we performed in vitro deubiquitination assay using purified ASXM2-WT/BAP1 or ASXM2-KR/BAP1.

Results: Importantly, we found that ASXL2 is monoubiquitinated on K370 of its ASXM domain and that monoubiquitination occured only in presence of BAP1. Strikingly, the K370R mutant is almost completely unable to promote BAP1 DUB activity, in contrast to the WT ASXM2 which promotes H2Aub deubiquitination. Thus, ASXM mono-ubiquitination is required for BAP1 DUB activity towards H2Aub in vitro and in vivo. Following viral transduction and chemicals treatments, we found that ASXL2-WT is less stable than the corresponding K370R mutant. Moreover, our screen data shows that monoubiquitination of ASXM2 is directly catalyzed by UBE2E family of ubiquitin conjugating enzymes. Using gain and loss of function studies we showed that BAP1, and ASXL2 play critical roles in the coordination of cell cycle progression. Notably, overexpression of ubiquitin mutant ASXL2 K370R abolishes normal cell proliferation.

Conclusion: Our results indicate that, through monoubiquitination, BAP1 and ASXL2 exert a tightly controlled regulation on cell proliferation, and provide a critical mechanistic link between H2A deubiquitination by BAP1/ASXL2 complex and tumorigenesis.

P20

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

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Keywords: leukemia, BRPF1, MOZ-TIF2, acetylation

Background information: Over 500 epigenetic regulators have been identified in humans raising an interesting question about how epigenetic dysregulation contributes to different diseases. Bromodomain and PHD finger-containing protein 1 (BRPF1) is a multivalent epigenetic regulator interacting with three histone acetyltransferases (HATs). The monocytic leukemia zinc finger protein (MOZ, also known as KAT6A) is one of the three HATs firstly identified in acute myeloid leukemia (AML). The KAT6A gene is rearranged with the chromosome inversion inv(8)(p11;q13), generating KAT6A-TIF2 (transcription intermediary factor 2) to confer leukemic stem cell (LSC) properties such as self-renewal to committed hematopoietic progenitors and consequently promotes leukemia development. It has been shown that KAT6A is required for maintaining the self-renewal of hematopoietic stem cells (HSCs). On the other hand, BRPF1 are often mutated in childhood leukemia and adult medulloblastoma. The mutations appear to disrupt the function of BRPF1, suggesting its tumor-suppressor role. However, it remains unclear how BRPF1 is linked to human hematopoietic development and leukemia.

Purpose of the study: We have recently reported that BRPF1 is essential for mouse hematopoietic stem cells. We hypothesize that BRPF1 regulates activities of KAT6A-TIF2 to confer LSC properties. Thus, my research interest is to investigate the role of BRPF1 in leukemic development and to develop new prognostic markers, personalized therapeutic targets markers, and drugs for early detection, clinical care, and treatment of pediatric or young adult leukemia.

Methods: In vitro histone acetylation assays were performed to exam the acetylation of KAT6A-BRPF1 complex. In addition, we will employ colony-formation assays with transduction of KAT6A-TIF2 in mouse bone marrow cells to determine whether BRPF1 is required for KAT6A-TIF2 to confer self-renewal to committed progenitors. In vivo, we will perform bone marrow transplantation assays to assess how BRPF1 collaborates with KAT6A-TIF2 to induce LSC properties.

Results: It has been reported that histone H3K23 acetylation (H3K23ac) is abundant in two human cell lines. We found that H3K23ac significantly lost in mouse Brpf1 KO bone marrow and spleen. In human, H3K23ac reduces in lymphoblastoid cell lines (LCLs) with BRPF1 mutations. Moreover, in vitro histone acetylation assays show that three BRPF1 cancer-related mutations fail to activate H3K23 acetylation, suggesting BRPF1 is required for H3K23 acetylation. We constructed KAT6A-TIF2 plasmid and will analyze how BRPF1 interacts with KAT6A-TIF2.

On the other hand, we have tested some histone deacetylase inhibitor (HDACi) and there are four HDACi including VPA (Valproic acid sodium salt), SAHA, TSA (Trichostatin A), and SB (Sodium Butyrate) show the ability to rescue the loss of H3K23ac in Brpf1 KO MEFs. This finding provides an important direction for further R&D of a new drug for leukemia treatment involved in the dysregulation of H3K23 acetylation.

Conclusion: In summary, this study gains insight into the role of an epigenetic regulator BRPF1 in leukemia. We found that BRPF1 is required for H3K23 acetylation catalyzed by histone acetyltransferase KAT6A. H3K23ac significantly reduces in human lymphoblastoid cell lines (LCLs) with BRPF1 mutations. Results from HDACi treatment indicate that VPA and SAHA have the potential role in treatment of pediatric or young adult leukemia.

P21

Title: Define the surfaceome landscape of hematopoieitic stem cells and pediatric leukemia specimens to improve the development of novel therapies for hematological diseases

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Keywords: Leukemia, Hematopoietic Stem Cell (HSC) transplantation, Surfaceome, HSC surface markers

Background information: Despite great advances in understanding the pathogenesis of various types of adult and childhood leukemias, there has been little progress in the development of new therapies. Over the past decades, hematopoietic stem cell transplantation (HSCT) isolated from umbilical cord blood (CB) has evolved as a potent curative treatment intervention for patients with different type of blood disorders. 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. The group of Dr. Guy Sauvageau and their collaborators have recently discovered a small-molecule, UM171, which stimulates the ex vivo expansion of CB HSCs. However, the lack of reliable surface markers that can prospectively identify HSCs is still a major hurdle for the optimization of CB grafts.

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

Methods: To discover novel and reliable HSC 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, which is responsive to UM171-induced cell expansion. Interestingly, we found that UM171 promotes the upregulation of many cell surface proteins, including the endothelial protein C receptor (EPCR) which we recently characterized as a novel HSC surface marker. 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, our published and preliminary data demonstrate the great potential of our surface proteomics method to identify novel and reliable HSC markers. The availability of the surfaceome landscape of HSCs will undeniably open new avenues for the optimization of HSCT. In addition, our potent surfaceomics approach will offer new opportunities for the development of antibody-based immunotherapies by uncovering targetable surface proteins from primary leukemia specimens.

P22

Title: Targeted clearance of senescent cells towards improving cancer therapy

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Keywords: Anti-cancer therapy, senescence, leukemia and lymphoma, cell death, translation.

Background information: Radiotherapy and chemotherapeutic agents target proliferating cells in a non-specific manner, triggering cellular senescence in both normal and cancer cells. Senescent cells remain metabolically active but are refractory to mitogenic signals. Although the senescence program blocks the proliferation of potentially transformed cells, the secretion of numerous pro-inflammatory cytokines, growth factors and metalloproteases (called the Senescence-Associated Secretory Phenotype or SASP) by senescent cells is deleterious for the surrounding tissues. Accumulation of senescent cells persists following anti-cancer therapy, favoring both local and systemic inflammation and contributing to the adverse effects of therapy including bone marrow suppression, cardiac dysfunction, cancer recurrence and fatigue. Hence, studies on survivors of childhood cancers such as leukemia suggest that anti-cancer therapy can cause long-term side effects usually related to aging. Interestingly, cell death induction of senescent cells in an engineered mouse model prevented the detrimental effects of chemotherapy.

Purpose of the study: We propose to characterize, both in vitro and in vivo, a novel family of senolytic compounds (a chemical triggering cell death of senescent cells specifically while sparing proliferative cells) in order to alleviate the secondary adverse effects and improve efficacy of anti-cancer therapy.

Methods: Specific elimination of senescent cells is challenging as elevated levels of the anti-apoptotic proteins of the BCL2 family provide senescent cells with an intrinsic resistance to apoptosis. Since the expression of some of these anti-apoptotic proteins relies on the translation initiation factor eIF4A, senescent and proliferating cells were treated with increasing doses of eIF4A inhibitors and cell survival was evaluated using flow cytometry-based assays. Western blotting and RT-qPCR were performed in cells treated with the compounds to analyze anti-apoptotic protein expression.

Results: We show that inhibition of eIF4A functions using small molecules of the rocaglate family triggers cell death in senescent cells while sparing proliferative cells. We further demonstrate that rocaglates decrease Bcl-xL and Bcl-w levels under conditions that trigger apoptosis in DNA damage-induced senescent cells. We now intend to characterize the molecular pathways responsible for the rocaglate-mediated apoptosis induction in senescent cells and to validate the senolytic potential of rocaglates in vivo in a lymphoma mouse model.

Conclusion: We expect our study to provide a proof of concept for the use of new senolytic drugs in combination with existing treatment options to improve the efficiency of anticancer therapy and dampen the burden of therapy-associated secondary effects affecting particularly patients treated during childhood.

P23

Title: Novel functions of nuclear eukaryotic translation initiation factor (eIF4E) in leukemia

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Keywords: Cancer, eIF4E, RNA processing, RNA export, translation

Background information: Eukaryotic translation initiation factor 4E (eIF4E) is highly upregulated in M4/M5 subtypes of AML and subsets of lymphoma. In fact, 30% of malignancies are known to have elevated eIF4E. In the nucleus, eIF4E targets approximately 3500 transcripts, many involved in cellular proliferation and tumor progression, for mRNA export. In addition, nuclear mRNA export targets contain a sequence element, 4ESE, which is recognized by eIF4E in the nucleus. RNA processing is a central regulatory stage in eukaryotes that impacts the ability of the cell to translate RNA into proteins, thereby bridging the gap between the transcriptome and proteome. Alternate polyadenylation (APA) is the use of alternative cleavage sites and/or alterations in the length of the polyA tail. This can have major impacts on the protein product by changing the ability of the RNA to proceed with export and/or the essential properties of the protein such as missing essential regulatory domains.

Purpose of the study: Here we propose that alternate cleavage and polyadenylation in response to up-regulated eIF4E will contribute to oncogenic activity. We further aim to 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 (4ESE), in both vector controls and eIF4E overexpressing cells (eIF4E-FLAG). Cells were fractionated into nuclear and cytoplasmic components. We implemented QuantSeq 3' end sequencing to generate nuclear libraries from Vector and eIF4E-FLAG (+/- 4ESE) nuclear samples for 3' end NGS. QuantSeq 3' is an innovative technology that allows for single nucleotide mapping of APA genome wide. It will determine APA and its spatial position relative to 4ESE sites. Validation cleavage assays were performed via RTqPCR with primers designed to flank the 5' and 3' end of the regulatory region, in the nuclear fraction. We further performed immunoprecipitation (IP), export assays, and RNAi to identify key co-factors in this process.

Results: (1) Elevation of eIF4E levels increases cleavage of endogenous targets cyclin D1and myeloid cell leukemia 1 (MCL1) as well as of a model mRNA containing only the minimal requirements for APA (LacZ +4ESE +PAS), (2) eIF4E elevation alters PAS site usage in model RNA constructs (3) eIF4E interacts with important RNA processing co-factors, in the nucleus, including the cleavage endonuclease CPSF3, and (4) eIF4E elevation modifies the expression of these and several additional co-factors. Our latest preliminary data suggest that elevated eIF4E impacts and potentially interacts with several additional key APA factors, including pre-mRNA processing protein, WDR33, RNA encoding cleavage factors, CPSF1&CPSF2, known oncogenic protein, FIPL1, and cleavage stimulation factors CSTF2 and symplekin.

Conclusions: Therefore, eIF4E could regulate expression of key co-factors of the RNA processing machinery, by effecting the processing of a broad array of mRNA transcripts and their proteins. We have shown that eIF4E is involved in RNA 3' processing given the

evidence suggesting eIF4E interacts with regulators of the process. These are first studies linking eIF4E and APA in cancer. This suggests a novel eIF4E driven APA pathway, which could contribute to AML and other cancers.

P24

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 defining novel therapeutical approach(es) 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 P-TEFb 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 P-TEFb 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 P-TEFb 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 fetal liver cells 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 (ChIP-qPCR). 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 Ikaros -/- influences the recruitment of NuRD complex, P-TEFb 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-P-TEFb complex in regulating XRN2 activity, we will introduce Ik6 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 PT-EFb 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-P-TEFb are interacting with TTF2 and are recruited simultaneously at the gene TSS. Furthermore, they are recruited with XRN2 at TTS of TAL1 and E2F4 genes. Finally, the study of expression of these genes in G1E2 and in fetal liver cells suggests that Ikaros influences the transcription termination of these genes. In fact, when Ikaros is absent, we detect readthrough transcripts after TTS of the genes, suggesting a defect in transcription 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 whether Ikaros can influence transcription termination. Ikaros would then be a unique tissue-specific transcription factor involved in the three steps of transcription. Whether the Ikaros mutation could affect transcription termination and thereby, expression levels of specific tumor suppressors or oncogenes in pediatric B-ALLs is not known. This study will provide novel information on the precise role of Ikaros and thereby, help to define new therapies for the treatment of hematopoietic disorders characterized by the mutation of Ikaros.

P25

Title: Tackling refractory/relapsed childhood cancer, one patient at a time

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Keywords: Oncology, Genomics, Actionable targets, Childhood

Background information: Childhood cancer is a group of heterogeneous complex diseases and remains the first cause of death among children in Western countries. The lack of therapeutic advances for refractory/relapse patients is even more worrisome 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: TRICEPS study, initiated on April 2014 at the Sainte-Justine UHC (Montreal, Canada), was shown to be a major breakthrough by offering 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 from biopsy to a detailed tumour analysis report.

Methods: Patients who were less than 18 years at initial diagnosis and who had refractory or relapsed cancer were deemed eligible for the study. WES of both tumour (300x) and normal DNA was performed together with the tumour RNA-seq (150M reads) on the Illumina Hiseq 2500 system (paired-end 100 bp, rapid mode) at the SJUHC's Integrated Clinical Genomics Centre. Individual tumor analysis was performed using a highly automated pipeline that combines several third-party software. The patient's specific dataset and the identification and validation of actionable driver mutations were interpreted by the TRICEPS multidisciplinary tumour board and targeted therapy was proposed for patients when possible.

Results: Through TRICEPS study, we initiated a personalized medicine program and demonstrates that identification of actionable alterations in paediatric tumors through clinical tumor profiling leading to individualized cancer therapy recommendation is feasible and effective at a single site and without variability in approaches in the context of our mono-institution research protocol. Over a period of 3 years, 60 relapsed/refractory cancer patients underwent extensive genomic investigation (exomic and transcriptomic sequencing). In all 60 patients analysed, we have identified clinically relevant relapse-specific mutations (SNVs, indels, fusions, CNAs) that could influence patient management and providing options for personalized interventions. We also assessed the functional impact of some of these cancer-specific alterations (Khater et al., 2017, Khater et al., 2018), such as a recurrent pediatric alteration resulting of an addition of three amino acids occurring in the kinase domain of the BRAF oncogene that confers resistance to RAF inhibitors and strongly suggesting it as a guide for treatment strategies.

Conclusion: TRICEPS results clearly influence further treatment strategies, diagnosis and/or outcome. This study shows real-life delays and problematics to overcome and think ahead in future settings and emphasize the need for more clinical trials of targeted therapies in pediatric patients with cancer.

P26

Title: Characterization of Dimer-Activated MLL and Probing the small GTPase Signaling Landscape in MLL-rearranged Cells

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Keywords: acute leukemia, mixed-lineage leukemia, structural biology, GTPases

Background information: Chromosomal rearrangements to the mixed lineage leukemia (MLL) gene result in aggressive infant leukemias. A recently published study demonstrated that the self-association (dimerization) of MLL has proven to be an activating mechanism for the MLL-AF6 rearrangement, enabling the fusion to specifically interact with gene expression proteins such as the DotCom epigenetic modulators and components of SEC. Critically, abrogation of dimerized MLL-AF6 was shown to inhibit leukemogenesis in mice. MLL-AF6 and other MLL fusions promote epigenetic and signaling changes, including promoting the activation of small GTPases (such as Ras/Rac) implicated in cancer progression and cell survival.

Purpose of the study: The aims of this study include providing a detailed structural characterization of the dimerization interface of MLL-AF6 and other selected MLL fusion partners. Additionally, real-time nuclear magnetic resonance (NMR) spectroscopy assays will be developed to monitor how cells harboring MLL-rearrangements perturb the activities of key small GTPases.

Methods: Plasmid constructs encompassing the fusion junctions of MLL-AF6 and other cytoplasmic protein partners predicted to self-associate are designed. These constructs can be expressed and purified in Escherichia Coli, can form dimers in solution, and have been used in crystallography screens. Upon successful crystallization, the structure of the MLL-fusion will be solved using X-ray crystallography, providing the first insights into the MLL-dimer interface.

Novel strategies using real-time (RT) NMR spectroscopy have been developed to quantitate GTPase activation. Importantly, as proteins of interest are isotopically labeled to make them NMR "visible", these experiments can be performed in the presence of whole cell lysates from MLL-rearranged cell lines. Using selective isotopic-labelling approaches, multiple GTPases (Ras and/or Rho family) can be monitored concurrently.

Results: Regions consisting of the MLL-AF6 fusion and dimerization interface have been successfully expressed and purified recombinantly. Native-PAGE demonstrates that AF6 forms dimers when fused next to MLL exon9 but not when fused next to exon10, correlating well with the atypically frequently observed exon9–AF6 fusions. Extensive crystal screening with our MLL exon9-AF6 construct has provided an initial hit which will be further optimized and scaled up to produce a suitable crystal for solving the structure. For NMR studies, multiplexed kinetic assays have been successfully developed using Rho and Ras family GTPases. Kinetics have been measured in the presence of GTPase cycle regulatory proteins such as GTPase activating proteins and guanine exchange factors as well as in the presence of cell extracts.

Conclusion: Dimerization could be a common biophysical mechanism of activation for MLL-fusions with cytoplasmic protein partners. Successfully solving the crystal structures of these fusion proteins will provide therapeutic avenues to perturb their

self-association. With the successful development of RT-NMR multiplexed assays in in vitro settings, assays can now be performed in cell lysates from MLL-rearranged cell lines to decipher how they affect the activation of small GTPases implicated in proliferation/migration.

P27

Title: Transcriptomic landscape of acute promyelocytic leukemia reveals aberrant surface expression of the platelet aggregation agonist Podoplanin

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

Keywords: Acute promyelocytic leukemia, podoplanin, bleeding

Background information: Acute promyelocytic leukemia (APL) is a subgroup of acute myeloid leukemia (AML) characterized by the t(15;17) chromosomal translocation resulting in the PML-RARA fusion gene. APL is a medical emergency because of associated subgroup-specific lethal early bleedings, but the mechanisms underlying these complications are still not completely elucidated.

Purpose of the study: We aimed to identify and describe novel determinants of APL-related bleedings.

Methods: RNA-sequencing of 30 APL and 400 AML samples was performed as part of the Leucegene project. PDPN overexpression and assessment by flow cytometry, platelet aggregometry, chemical screening, xenotransplantation and bleeding times were performed as described in Lavallée VP et al, Leukemia, 2018.

Results: Using a comparative RNA-sequencing analysis, we 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 test, we established that PDPN represents the most specific surface marker in APL, and that this test could represent a complementary diagnostic approach to rapidly identify patients.

Intriguingly, PDPN protein expression is highly variable between APL patients (range from < 2% to 89% of positive cells by flow cytometry). This variation is also reflected in the transcriptome as the proportion of PDPN-expressing promyelocytes strongly correlates (r = 0.89) with gene expression levels. Notably, the homeobox-containing transcription factors IRX1, UNCX, SIX3 and NKX3-2 are also specifically expressed in APL compared to other leukemia subgroups and to normal promyelocytes, and their expression correlates with that of PDPN in APL cells, possibly indicating transcriptional filiations. This hypothesis is currently under investigation by: i) PML-RARA overexpression in cell lines, ii) bulk RNA sequencing analysis in sorted populations of primary APL specimens based on PDPN expression and iii) single-cell RNA-sequencing.

In line with PDPN role in hemostasis during embryogenesis, engineered overexpression of PDPN in human leukemic cells caused aberrant platelet binding, activation and aggregation, properties that we also observed in PDPN-expressing, but not in PDPN-negative, primary leukemias. Finally, engineered PDPN expression on leukemic cells in a xenograft model was distinctively associated with thrombocytopenia and resulted in prolonged bleeding time in vivo.

Conclusion: Aberrant PDPN expression on APL cells contributes to thrombocytopenia and represents a novel determinant of APL-induced bleedings. Next efforts will aim at better understanding PDPN regulation in this disease.

P28

Title: Modulation of T cell function by CD271

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Keywords: 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 investigate the immunoregulatory role of CD271.

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. Additionally, to determine the physiological role of CD271 on T cell modulation, we co-cultured antigen-specific mouse T cells with artificial antigen presenting cells overexpressing CD271. 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, in autoimmune model and in the presence of chronic antigen challenge 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, our co-culture model demonstrated that proliferation of these T cells is partially inhibited in the presence of artificial antigen presenting cells overexpressing CD271. We are expanding these results to include additional cellular models. Interestingly, 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 control chronic antigen challenge more efficiently as compared to control mice.

Conclusion: Together, these results suggest that CD271 modulates T cell function. We are working to determine the immunosuppressive or immune privilege properties of immunoregulatory cells expressing CD271.

P29

Title: Targeted screening for novel genetic driver mutations that elicit resistance to chemotherapy in Burkitts lymphoma using a novel CRISPR-based site-specific mutagenic approach

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Keywords: Lymphoma, Burkitts, Chemoresistance, CRISPR/Cas9, CRISPR-X, Driver Mutations, Genetic Screens

Background information: NHL is the 6th most commonly diagnosed cancer in Canada, comprising ~4% of all new diagnoses. Among NHLs, BL is a unique form, characterized by its aggressiveness and its predominance in children and young adults (~30% of all new pediatric lymphoma cases). Like most NHLs, BLs originate from germinal centers, which serve as hotspots for B-cell expansion and diversification, in part through the controlled use of somatic DNA hypermutation and genomic rearrangements that alter the immunoglobulin (Ig) genes. Accordingly, BL emerges through miscues stemming from these very processes, which lead to frequent genomic translocations, cytogenetic alterations and somatic mutations, that notably includes the chromosomal translocation t(8;14) which brings MYC under control of the Ig locus rendering it oncogenic. While most cases of BL are highly treatable with short and intensive combination chemotherapeutic regimens, with significant long-term remission rates, a significant portion of BL cases (up to 40%) relapses or becomes refractory to treatment and usually succumb to the disease within 2 months. While the biology of de novo BL genetic drivers is fairly well characterized, the genomic landscape of rrBL has yet to be reported.

Purpose of the study: Our collaborator, Dr. Nathalie Johnson, has recently sequenced biopsies from two rrBL patients and found many de novo mutations that were enriched following relapse, several of which had never been reported previously in BL. Our overarching goal is to characterize these mutations in an in vivo CRISPR/Cas9-based functional assay in order to ascertain their impact in their resistance to chemotherapy. In addition to distinguishing drives from passenger mutations that contribute to rrBL, such research can provide valuable insight into novel biomarkers that will aid to prioritize high-risk BL patients that would benefit from alternative therapy. And more broadly, this project may have implications for other chemoresistant lymphoma subtypes.

Methods: In order to generate these genetic mutations in vivo, we use a novel version of CRISPR/Cas9, termed CRISPR-X, that fuses an endonuclease-dead Cas9 (dCas9) to a hyperactive mutant of cytidine deaminase AID, which promotes the mutagenesis of regions adjacent to its cognate sgRNA. We use custom libraries of sgRNAs that target mutagenic sites associated to chemoresistance in patients, in a pool-based enrichment functional genetic screen in in vitro cell lines and in in vivo in the Eμ-myc mouse model.

Results: As a proof of concept experiment, we generated a library of CRISPR-X based sgRNAs that spanned all known mutational hotspot sites of Trp53 and tested the robustness of the CRISPR-X to elicit resistance to Nutlin-3a, a known activator of the p53. In in vitro cell assays,

several (but not all) sgRNAs elicited resistance to the drug, demonstrating the feasibility of this functional assay approach. Following these initial observations and guidelines,

we generated a custom library of over ~250 sgRNAs targeting both patient-derived and literature curated de novo mutations. Current progress is ongoing to adapt this system for use in the Eμ-myc lymphoma mouse, a chemoresponsive mouse model that offers a unique clinically relevant setting to functionally interrogate their clinical relevance.

Conclusion: Altogether, these results validate the use of a CRISPR-X pool-based screening approach for the identification of both novel gain and loss-of-function mutations observed in rrBL patients, leading to potential novel druggable pathways and biomarkers to better stratify patients to overcome resistance to standard of care chemotherapy.

P30

Title: Generation of a Mouse Model of Acute Megakaryoblastic Leukemia

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Keywords: Pediatric AMKL, fusion oncoproteins, mouse model, cellular signalling, therapeutic targets

Background information: Pediatric acute megakaryoblastic leukemia (AMKL) is an aggressive disease associated with poor outcome, and current protocols for treatment of AMKL have proven unsuccessful. Transcriptome, whole-genome sequencing, FISH and RT-PCR analyses of non-Down Syndrome (DS) AMKL pediatric patients recently revealed the frequent occurrence of a cryptic oncogenic fusion, CBFA2T3-GLIS2, in the disease. Pediatric AMKL patients carrying the CBFA2T3-GLIS2 gene fusion display increased resistance to chemotherapy and manifest a distinct gene expression profile compared to other pediatric AMKL patients. Furthermore, mouse hematopoietic stem and progenitor cells (HSPC) transduced with CBFA2T3-GLIS2 gene fusions display enhanced proliferation and self-renewal capacities. Despite these observations, however, the role of CBFA2T3-GLIS2 in the transformation of hematopoietic cells remains poorly characterized.

Purpose of the study: We propose to generate a CBFA2T3-GLIS2-driven AMKL mosaic mouse model in order to i) identify genes involved in the maintenance and progression of AMKL cells, and ii) validate therapeutic targets of CBFA2T3-GLIS2 AMKL in vivo.

Methods: We co-transduced HSPC harvested from E13.5-14.5 C57BL/6 mouse fetal liver with a retroviral vector encoding for a mCherry-labeled CBFA2T3-GLIS2 oncoprotein and a vector encoding for Luciferase to monitor leukemogenesis via peripheral blood sampling and bioluminescent imaging. HSPC expressing CBFA2T3 -GLIS2 were introduced into lethally irradiated (8Gy) syngeneic recipients (B6SJL) via tail vein injection. Disease progression was followed by peripheral blood analysis using flow cytometry and bioluminescence monitoring over the course of 6 months following injection. Bone marrow and spleen of leukemic mice were isolated and phenotyped for enrichment of the megakaryoblast/megakaryocyte population (e.g. CFU-meg, Lin-Sca-1+ c-Kit+ {LSK}, CD150+, CD34-, CD41+/CD61+).

Results: AMKL was generated with a penetrance of 50-60% and affected mice displayed striking splenomegaly. Our model recapitulates many features of the human disease that is characterized by an accumulation of megakaryocytic blasts in the peripheral blood and bone marrow as well as infiltration into extramedullary tissues such as the spleen and liver. Blasts also express one or more megakaryocyte markers (CD61, CD41, CD42, factor VIII), and the morphology of bone marrow and spleen cells is consistent with megakaryoblasts and megakaryocytes.

Conclusion: Current protocols for treatment of acute myeloid leukemia have proven unsuccessful with children with CBFA2T3-GLIS2 positive AMKL. Furthermore, the CBFA2T3-GLIS2 oncogenic fusion is restricted to pediatric non-DS AMKL and characterized by its aggressiveness. The prognosis of children with this fusion is significantly worse when compared to CBFA2T3-GLIS2-negative patients in general. This is likely due to lack of understanding of the specific molecular mechanisms by which

CBFA2T3-GLIS2 drives tumorigenesis and sustains self-renewal. Hence, by i) uncovering genes involved in the development of the disease and ii) identifying novel therapeutic targets leading to growth suppression of CBFA2T3-GLIS2 AMKL cells, this project will directly support the advance of optimized and efficient clinical protocols specific to this life-threatening subset of pediatric AMKL.

P31

Project Title: Whole genome CRISPR/Cas9 knock-out chemogenomic screens in a B-cell lymphoma cell line reveal specific drug mechanism-of-action and synergistic interactions

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Advisor: Michael Tyers, IRIC

Keywords: CRISPR/Cas9, chemogenomic screens, B-cell lymphoma, synergistic chemical probe effects, drug resistance

Background: Gene loss-of-function may confer sensitivity or resistance to relevant drugs and chemical probes. The detection of negative genetic interactions, characterized by increased vulnerability to the cognate chemical substance, offers compelling avenues towards the synergistic eradication of cancer. Complex genetics in the latter suggest that the alternative pursuit of positive genetic interactions might reveal the underlying cause for drug resistance.

Purpose of the study: Generate a chemogenomic profile matrix using a chemical probe library targeting various cellular processes such as growth, replication, DNA damage, mitosis, metabolism, epigenetics, and signaling.

Methods: A custom genome-wide CRISPR/Cas9 knockout library in a B-cell lymphoma cell line was exposed to the chemical substance collection for 8 days, genomic DNA was extracted, processed and high-throughput sequenced. The relative abundance of sgRNAs in the sample informs on whether the loss of a specific gene was detrimental or advantageous for proliferation in the presence of the chemical compound.

Results: The observed changes in sgRNA abundance, termed chemical-genetic interactions, recapitulated known pathways, revealed unexpected genetic targets for known drugs, and predicted mechanism-of-action for uncharacterized compounds. Inhibitors with the same target revealing different chemogenomic profiles were interpreted to display different off-target effects and/or different mechanisms of action.

Conclusion: We have generated a genome-wide CRISPR/Cas9 knockout library in a B-cell lymphoma cell line to expose genetic vulnerabilities to chemical compounds. This approach has allowed us to find promising candidates for synergistic drug treatments, which we are currently further exploring. In addition, we have revealed a great number of positive genetic interactions where the loss of a specific gene results in drug resistance. Altogether, this approach has proven itself as a valuable tool to elucidate compound mechanism and gene function.

P32

Title: High expression of SPAG1 is associated with a poorer clinical outcome in intermediate risk acute myeloid leukemia that can be partially overcome by hematopoietic stem cell transplantation

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Keywords: Acute myeloid leukemia, Biomarker, Gene expression, Prognosis

Background: Acute myeloid leukemia (AML) is a heterogeneous disease with high mortality rate. After achieving complete remission (CR) with chemotherapy, patients are treated with a consolidation treatment to prevent relapse. The type of consolidation is determined by risk stratification based on genetic analyses of leukemic cells and by clinical factors. Patients with favorable risk are treated with chemotherapy whereas patients with adverse risk are referred for a hematopoietic stem cell transplantation (HSCT). Patients in the intermediate risk category (~40%) represent a clinical dilemma regarding consolidation. Consequently, new prognostic tools are needed to refine the risk stratification of this patients' subgroup.

Purpose: This study aims to identify a biomarker to improve risk stratification of patients with intermediate risk AML and to identify who will benefit of HSCT.

Methods: We analyzed RNA sequencing data of 165 specimens from patients with de novo intermediate risk AML treated with curative intent. Data from 24 586 genes were normalized as RPKM values and underwent logarithmic transformation and standardization. Cox proportional hazard models were used to assess the prognostic impact of gene expression (GE) on overall survival (OS) and relapse-survival (RFS). We performed univariate and multivariate analyses adjusted for age and white blood cell count (WBC) at diagnosis, mutations in NPM1, FLT3, CEBPA, RUNX1, ASXL1, TP53 and DNMT3A and HSCT as a time-dependent (TD) covariate. Interaction between GE and TD-HSCT was tested in multivariate models for OS and RFS. Genes for which the interaction test had a significance level of < 0.10 were retained for further analyses. GE values of potential candidates were dichotomized and their prognostic impact was reassessed as binary variables. Tests were two-sided with a significance level of < 0.05.

Results: We identified SPAG1 as the gene with the highest hazard ratios (HR) for OS and RFS in multivariate analyses. SPAG1 expression was dichotomized on the median RPKM value. Patients with high expression of SPAG1 had similar age, WBC at diagnosis, and HSCT frequency compared to patients with low expression of SPAG1, but had a higher frequency of FLT3-ITD and DNMT3A mutations (both $p = 0.02$). In univariate analyses, SPAG1-high patients had poorer OS ($HR = 1.75$, $p < 0.01$) and RFS ($HR = 1.90$, $p < 0.01$) when survival times were censored at time of HSCT. High expression of SPAG1 remained significantly associated with OS ($p = 0.01$) and RFS ($p < 0.01$) in multivariate models. In the RFS model, there was an interaction between SPAG1 GE and TD-HSCT ($p < 0.09$). SPAG1 had a lower prognostic impact when we included TD-HSCT in the model ($HR\ 1.65$, $p = 0.02$) than when we censored survival at time of HSCT ($HR = 2.34$, $p < 0.01$).

Conclusion: In patients with intermediate risk AML, high expression of SPAG1 is associated with poor OS and RFS. The impact of SPAG1 expression on RFS is lower when adjusted for HSCT indicating that the poor prognosis conferred by high expression of this gene may be partially overcome by HSCT.

P33

Title: Optimizing the T cell repertoire for adoptive immunotherapy

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

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 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 murine and human 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 biases in differentiation, effector function and persistence among naive T cells, murine CD4+ T cells 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 assessed cytokine production by antigen specific CD5lo and CD5hi CD4+ T cells after acute antigen challenge in vivo (pathogen infection or dendritic cell vaccination). Further, to determine if CD5 levels mark comparable T cell populations in humans as in mouse, we assessed regulation of CD5 levels throughout human T cell development and during T cell activation. In addition, we sorted CD5lo and CD5hi CD4+ T cells from peripheral blood mononuclear cells (PBMCs) and evaluated expression of genes which are known to be differentially expressed in murine CD5lo/hi CD4+ T cells and assessed their cytokine production after in vitro activation.

Results: Our results show that in vitro activated murine CD5lo CD4+ T cells produce relatively greater amounts of the Th1 cytokine IFNγ compared to their CD5hi counterparts. Further, antigen-specific polyclonal CD5lo CD4+ T cells also display increased cytokine production including IFNγ, IL-2 and TNF-α, at the peak of the immune response to a number of acute antigen challenges. Mechanistically, this differential cytokine production is independent of TCR affinity as adoptively transferred TCR transgenic CD5lo CD4+ T cells also produce more cytokines compared to their CD5hi counterparts during acute antigen challenge. Further, our preliminary data shows that CD5lo CD4+ T cells predominate in chronic antigen stimulation models, as is the case in cancer. Importantly, we show that similar to mouse, CD5 levels on human T cells are regulated by the strength of T cell activation and that functionally, human CD5hi CD4+ T cells produce more IL-2 and display similar differences in gene expression as reported for murine CD5lo/hi CD4+ and CD8+ T cells.

Conclusion: Our data suggests that low CD5 expression on CD4+ T cells may be a predictive marker of T cell subsets with improved functionality and persistence for adoptive T cell therapy. Future studies are focussed on determining the in vivo potency of tumor-specific CD5lo and CD5hi CD4+ T cells.

P34

Title: Tracing Myc Expression for Small Molecule Discovery

Authors: Jutta Steinberger, Francis Robert, and Jerry Pelletier

Affiliation: Department of Biochemistry, McGill University

Keywords: Myc, small molecule inhibitors, Bufalin, phenotype-based screen, CRISPR

Background information: The deregulation of Myc expression is found in a wide array of human cancers. Myc is a pleiotropic transcription factor regulating the concerted expression of genes responsible for growth and expansion of somatic cells. Amplification or overexpression of Myc leads to an imbalance in the regulation of cell proliferation, depicting a key step in cancer onset and progression. The systemic inhibition of Myc through the dominant-negative Omomyc allele in tumor-bearing transgenic mice was shown to have a profound effect on various cancer types while having only mild and reversible effects on normal tissue. This makes Myc an interesting target for the pursuit of pharmacological strategies aimed at blocking Myc activity.

Purpose of the study: This study aims at identifying small molecule inhibitors of Myc expression in a high-throughput manner by tracing endogenous Myc expression and the subsequent characterization of their mode of action.

Methods: In order to identify modulators of Myc expression, it was important to assess Myc expression while minimally interfering with the Myc locus. To this end, we used CRISPR/Cas9 to engineer a JJN-3 cell line whereby Myc expression could be traced using an unstable variant of GFP (d2GFP, t1/2= 2h) (aka D11) in order to minimize the exposure time of cells to compound (5 hours). Using fluorescence readout as a surrogate marker for Myc expression, we screened ~10,000 compounds from FDA drug collection, bioactive compounds and in-house curated natural products (NP) collection. 115 hit compounds (1.15%) were identified, of which 52 remained after removal of duplicates, resupply and retesting. JJN-3 cells infected with a vector expressing d2GFP driven from the CMV promoter was used as counter-screen cell line. Compounds acting similarly in D11 and JJN-3/d2GFP cells were eliminated.

Results: Cardiac glycosides (CGs) made up a significant proportion (~30 %) of primary hits. Further characterization showed that their mechanism of action was linked to ATP1A1 function. Furthermore, CGs were shown to be potent inhibitors of Myc transcription and affect global translation by inducing the phosphorylation of eIF2α. A number of hits from the Bioactive compound collection indicated that inhibition of several key cellular processes feedback to affect Myc expression. Within the NP hits, we undertook activity-guided purification from two extracts and identified jasplakinolide, dolastatin-12, and tumonoate A as the active components in these extracts. Jasplakinolide induces actin polymerization to exert potent anti-proliferative effects towards tumor cells. Dolastatins induce mitotic arrest possibly by binding to tubulin and causing microtubulin depolymerization. Two more drugs that target actin and microtubules to prevent polymerisation were identified as hits, Cytochalasin D and dihydrorotenone. Taken together, these results indicate that we uncovered crosstalk between cytoskeletal integrity and Myc expression.

Conclusion: In conclusion, our results show the power of CRISPR/Cas9 technology to engineer phenotype-based assays to identify expression modulators from a specific genomic locus. We identified a number of compounds affecting various different key

cellular processes and consequently affecting Myc expression. Thus, our work reveals the magnitude of possibilities of how Myc expression can be influenced and taken advantage of for the therapy of Myc-dependent tumor types.

P35

Title: Integrin-α3 as a new marker for cord blood derived hematopoietic stem cells: redefining the hierarchy of cultured stem cells

Author: Elisa Tomellini, Post PhD 2017-2019

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Keywords: Hematopoietic stem cells, graft optimization, ex vivo expansion, UM171.

Background information: Hematopoietic stem cell (HSC) transplantation constitutes one of the most effective therapeutic strategies to treat numerous hematological diseases. Ex vivo expansion of HSCs, especially from human cord blood cells (CB), is therefore of great interest for clinical purposes and for genetic manipulation. Unfortunately, the biggest limitations in this context remain the absence of markers to univocally identify cultured HSC and the lack of understanding of the molecular mechanisms regulating stem cells self-renewal ex vivo. Those issues highly benefited from the discovery by our laboratory of the small molecule UM171 (promoting HSCs expansion ex vivo) and the identification of the Endothelial Protein C Receptor (EPCR) as one of the most reliable surface markers for cultured HSC.

Purpose of the study: The aim of this research project is the characterization of hematopoietic stem cells originated from expanded human CB in order to redefine the hierarchy of cultured HSC.

Methods and Results: Transcriptome analysis of EPCR+ cells allowed us to further improve the accuracy of prospective HSC identification in culture by the addition of the Integrin-α3 (ITGA3). ITGA3 expression at the top of the surface phenotype associated with human HSC (EPCR+CD90+CD34+CD45RA- subset) has shown to be sufficient to split this population in two functionally distinct fractions. ITGA3-positive cells, opposed to the ITGA3-negative fraction, exhibit robust multilineage repopulation and serial reconstitution ability in immunocompromised mice. Moreover, lentiviral-mediated ITGA3 knockdown is sufficient to compromise the long term (LT)-HSC phenotype in vivo of cultured HSC as well as their lineage potential. Gene expression profiling showed striking molecular similarity between ITGA3+ and ITGA3- cells, including some fundamental hematopoietic programs known to govern HSC specification and function. Nevertheless, our analysis uncovered unique differentially expressed genes that are candidates for governing HSC self-renewal.

Conclusions: Altogether, our results indicate that ITGA3 is a reliable marker for cultured HSC, narrowing the frequency of LT-HSC that can be isolated in culture. Deciphering the function of genes upregulated in primitive ITGA3+ HSCs will provide an invaluable resource for dissecting the genetic programs that govern hematopoietic stem cells biology.

P36

Title: BH3 profiling reveals dependency on multiple anti-apoptotic proteins in primary non-Hodgkin lymphoma samples

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

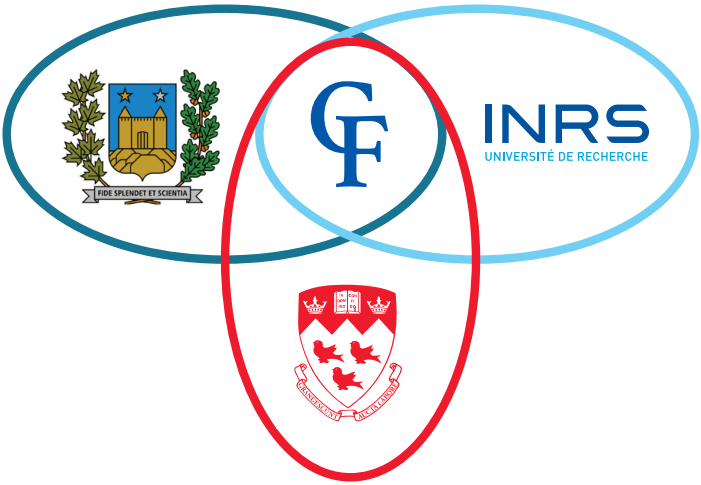
Background information: Lymphoma is the most common cancer in the adolescent and young adult population (18-39 years old), with diffuse large B cell lymphoma (DLBCL) being the most common non-Hodgkin lymphomas (NHL). DLBCL can be cured with chemotherapy but relapsed DLBCL is fatal in 90% of cases. A major cause of mortality in these patients is the co-expression of two proteins, MYC and BCL2, which synergize to promote cellular proliferation and inhibition of apoptosis. Venetoclax targets BCL2 and holds promise to improve the survival of these patients. However, it is active in only a subset of BCL2- expressing lymphomas. We hypothesize that BCL2 may not be the only apoptotic protein keeping the cells alive in these lymphoma subtypes.

Purpose of the study: Our aim is to determine the apoptotic blocks in primary NHL 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 chemotherapy. Understanding which patients are most likely to respond to venetoclax and how to increase the proportion of responders will result in better treatment options for this group of patients.

Methods: BH3 profiling specifically measures cytochrome c release by flow cytometry after cells are exposed to synthetic pro-apoptotic BH3 peptides. We can then assess the cell's response to these peptides and use the information to infer how easily the cancer cells could be killed. We perform this assay on primary samples (baseline profiling), as well as cultured cells that have been pre-treated with various chemotherapies (dynamic profiling) to determine if the cells' sensitivities to venetoclax are increased post chemo.

Results: We have performed baseline profiling on 165 primary samples. Overall, DLBCL is the most heterogeneous population, with significantly lower responses to 100μM BIM peptide than other lymphomas, and non-malignant controls. We also observe that the subset of DLBCLs that also have both a BCL2 and a MYC translocation, so called "double hit" high grade B cell lymphoma (HGBL-DH), respond significantly better to venetoclax than the other DLBCLs. For the lymphoma samples that do not have a good baseline response to venetoclax (e.g. DLBCL, FL), we have shown that combining venetoclax with an MCL1 inhibitor greatly enhances response, when compared to the effects of either compound alone. We have also tested cell lines, and have found that venetoclax-insensitive cells can become sensitized by pre-treating with doxorubicin or microtubule depolymerizing drugs.

Conclusion: Non-Hodgkin lymphoma is an extremely heterogeneous disease, with varying levels of baseline sensitivity to the BCL2 inhibitor venetoclax. We believe that venetoclax-insensitive lymphomas depend on both BCL2 and MCL1 for survival and that pre-treatment with components of the R-CHOP treatment regimen may overcome this block and improve venetoclax response.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