# Cole Foundation

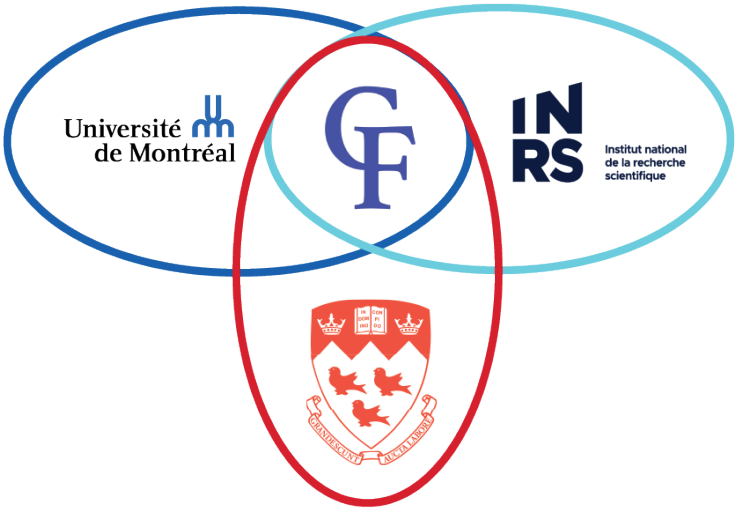
«Research Celebration Day»

**May 6, 2022**
16th Year

Journée «Célébrons la recherche»

# de la Fondation Cole

**6 mai 2022**
16e Année



## The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD programmes to promote research in pre-leukemia, leukemia, and other leukemia-related diseases in children and young adults, as well as the development of clinical care for patients affected by these diseases. With the announcement of 2019 Fellows, the Fellowship programme has supported more than 190 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 $12 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
- **Ms Nancy Wells** – Secretary/Treasurer
- **Dr. Michel Bouvier** – Board Member
- **Dr. Pierre Boyle** – Board Member
- **Dr. Pierre Chartrand** – Board Member
- **Ms. Gabrielle Cole** – Board Member
- **Ms. Viviane Cole** – Board Member
- **Mr. Charles Kaplan** – Board Member
- **Mr. David Laidley** – Board Member
- **Ms. Anne Lewis** – Board Member
- **Dr. Evan Lewis** – Board Member
- **Mr. Bruce McNiven** – Board Member

## La Fondation Cole

La Fondation Cole soutient la recherche sur la pré-leucémie, 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 2019, le programme a appuyé plus de 190 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 12 millions $ 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.

**La Fondation Cole**

- **M. Barry Cole** – Président
- **Mme Nancy Wells** – Secrétaire / Trésorière
- **Dr Michel Bouvier** – Membre du conseil d'administration
- **Dr Pierre Boyle** – Membre du conseil d'administration
- **Dr Pierre Chartrand** – Membre du conseil d'administration
- **Mme Gabrielle Cole** – Membre du conseil d'administration
- **Mme Viviane Cole** – Membre du conseil d'administration
- **M. Charles Kaplan** – Membre du conseil d'administration
- **M. David Laidley** – Membre du conseil d'administration
- **Mme Anne Lewis** – Membre du conseil d'administration
- **Dr Evan Lewis** – Membre du conseil d'administration
- **M. Bruce McNiven** – Membre du conseil d'administration

# Program

*10:00 a.m. - 12:00 p.m.*

### Fellowship Poster Exhibition

*12:00 p.m. - 1:00 p.m.*

### Lunch

*1:00 p.m. - 2:00 p.m.*

### New Voices – New Ideas

- **Dr. Vincent-Philippe Lavallée** – Research Centre of the Sainte-Justine University Hospital
  *Genetic predispositions and cellular progression in acute myeloid leukemias*
- **Dr. Martin Smith** – Research Centre of the Sainte-Justine University Hospital
  *Ultra-fast molecular diagnosis of leukemia*
- **Dr. Yoshiaki Tanaka** – Maisonneuve Rosemont Hospital Research Centre
  *Investigation of functional roles of a splicing variant of Cyclin E1 in leukemia*

*2:00 p.m. - 3:15 p.m.*

### Keynote speaker: Dr. Ari Melnick

*Epigenetic mechanisms inducing malignant transformation of the immune system*

*3:15 pm - 3:30 pm*

### New fellows and announcement of prizes

*3:30 pm*

### Reception

**Ari M. Melnick,** MD, is Gebroe Family Professor of Hematology/Oncology in the Departments of Medicine and Pharmacology at the Weill Cornell Medical College in NewYork City.

He is chair of the Hematologic Malignancies Program of the Weill Cornell Cancer Center, Director of the Sackler Center for Biomedical and Physical Sciences at Weill Cornell.

He has chaired a number of international meetings in hematology, cancer and epigenetics.

He has authored or co-authored more than 250 manuscripts in journals such as Nature, Science, Cell, Cancer Cell, Nature Medicine, Nature Immunology and the New England Journal of Medicine.

# THE 2022–2024 COLE FOUNDATION FELLOWS

## McGill University

■ **Chu-Han Feng,** PhD program

**Supervisor:** Michel Tremblay, Goodman Cancer Institute/Department of Biology, McGill University

**Project Title:** Modulating PTPN1 and PTPN2 in CAR-NK cell immunotherapy against acute myeloid leukemia in young adults

**Description:** Treating patients with relapsed or refractory (r/r) AML remains a challenge. Our research aims to generate an "off-the-shelf" immunotherapy for young adults r/rAML patients, through simultaneously modulating two non-receptor phosphatases PTPN1 and PTPN2 in chimeric antigen receptor engineered natural killer cells, to enhance their anti-tumor cytotoxic functions via cytokine sensitization.

■ **Bruktawit Maru,** PhD program

**Supervisor:** Maureen McKeague, Department of Pharmacology and Therapeutics, McGill University

**Project title:** Identification and Targeting of Predictive Biomarkers of Cytarabine Efficacy in Acute Myeloid Leukemia

**Description:** The non-response and relapse rates of acute myeloid leukemia (AML) pediatric patients treated with the first-line therapy cytarabine remains high. We are therefore identifying predictive biomarkers of cytarabine efficacy to better stratify patients for "salvage-like" therapy. Specifically, we are studying transcriptional biomarkers involved in the cytarabine-mediated cell death mechanism and developing RNA therapeutics to re-sensitize AML to cytarabine.

## Université de Montréal

■ **Mila Bjelica,** PhD program

**Supervisor:** Elie Haddad, CHU Sainte-Justine

**Title:** Engineering natural killer cells against cytomegalovirus and Epstein Barr virus

**Summary:** Complications from opportunistic viral infections, namely cytomegalovirus (CMV) and Epstein Bar virus (EBV) represent a major problem for pediatric blood cancer patients. This project aims to develop chimeric antigen receptor expressing natural killer cell-based therapies to treat CMV and EBV in these patients.

■ **Caroline Capdevielle,** Post PhD program

**Supervisor:** Laura Hulea and Frederick A. Mallette**,** Maisonneuve-Rosemont Hospital Research Center; Department of Biochemistry and Molecular Medicine, Université de Montréal

**Project title:** Characterization of metabolic rewiring and identification of potential therapeutic targets in *CBFA2T3-GLIS2*-dependent AMKL
**Description:** Transcriptomic analyses in mouse AMKL cells expressing CBFA2T3-GLIS2 (CG2) identified numerous genes pertaining to metabolism. We propose to investigate how metabolic rewiring associated to *CG2* gene fusions contribute to malignant transformation in order to identify and validate new metabolic therapeutic target in AMKL.

■ **Verena Gress,** PhD program

**Supervisor:** Sonia Cellot, CHU Sainte-Justine

**Project title:** Identification of small molecules for genotype-specific targeted therapies in high-fatality pediatric leukemia

**Description:** Pediatric acute megakaryoblastic leukemia (AMKL) is a rare subtype of acute myeloid leukemia (AML) with poor cure rates. In our lab we are able to model these leukemia with recurrent oncogenic fusions and are now working on identifying novel therapeutic vulnerabilities to improve patient outcome.

■ **Nafiseh Keshavarzian,** PhD program

**Supervisor:** Denis-Claude Roy and Dave Vibhuti, Maisonneuve-Rosemont Hospital Research Centre

**Project title:** Identify and characterize minor histocompatibility antigen (MiHA) specific T cell receptor

**Description:** This project will be to evaluate T cell expansion specific to several minor histocompatibility antigens (MiHAs) preferentially expressed on blood cell lineages and presented by several HLAs. The MiHA-recognizing T cell receptors will be isolated and cloned, and they will eventually be used to engineer primary T cells for immunotherapy against hematologic cancers.

■ **Banafsheh Khakipoor,** Post PhD program

**Supervisor:** Vincent-Philippe Lavallée, CHU Sainte-Justine

**Project title:** Characterization and comparison of CNS-infiltrating and bone marrow cells in pediatric AML patients

**Description:** Detection of leukemia cells that infiltrate the central nervous system (CNS) is critical and these cells remain poorly

understood. We will use single-cell RNA sequencing to detect CNS-infiltrating cells in pediatric leukemia patients and employ computational methods to study aberrant gene expression and regulatory networks in these cells.

- **Tibila Kientega,** PhD program

  **Supervisors:** Francis Rodier, Centre de recherche du CHUM-Institut du cancer de Montréal (CRCHUM-ICM) and Daniel Sinnett, CHU Sainte-Justine

  **Project title:** Assessment of cell senescence biomarkers during premature aging due to cancer treatment

  **Description:** Cancer therapy-induced accelerated aging and cellular senescence underlies aging-associated diseases. We aim to evaluate human childhood acute lymphoblastic leukemia (ALL) premature aging using senescence associated biomarkers and to test pharmacological ablation of senescent cells to delay age-associated phenotype in a mouse model of ALL therapy-induced senescence.

- **Louise Rethacker,** Post PhD program

  **Supervisor:** Elie Haddad, CHU Sainte-Justine

  **Project title:** Functional characterization of factors influencing natural killer (NK) cell activity against NKresistant **tumor models**

  **Description:** Mechanisms preventing the full cytotoxic anti-tumor activity of NK cells remain understudied. This project seeks to use whole-genome CRISPR screens in both NK and tumor cells to identify genes that, when inhibited, potentiate the cytotoxic activity of NK cells towards cancer cells and in turn improve NK cell-based immunotherapies.

- **Ali Smaani,** PhD program

  **Supervisor:** Jean-Sébastien Delisle, Centre de Recherche de l'Hôpital Maisonneuve-Rosemont

  **Project title:** Effet de l'expression de la Neuropiline-1 sur fonction des lymphocytes T CD8 dans le contexte du cancer et de la réponse du greffon contre la leucémie

  **Description:** Notre recherche porte sur le récepteur Neuropiline-1 dont l'expression est associée à de l'immunosuppression dans diverses populations de cellules immunitaires. Notre projet consiste à trouver des moyens de favoriser la réaction du greffon contre la leucémie tout en limitant la réaction du greffon contre l'hôte, et ce, afin d'améliorer le taux de réussite des greffes de cellules souches et de réduire le

risque de rechutes de la leucémie. Nous croyons que la modulation de l'expression de la Neuropiline-1 dans des populations spécifiques de cellules immunitaires pourrait aiguiller les réactions du greffon contre la leucémie et du greffon contre l'hôte.

- **Regina Strakhova,** PhD program

  **Supervisor:** Matthew James Smith, Department of Pathology and Cell Biology, Université de Montréal

  **Project title:** Biophysical characterization of oncogenic RAS signaling in pediatric leukemia

  **Description:** Mon projet de recherche se focalise sur l'étude des petites GTPases de la famille RAS. Avec l'aide de la Résonance Magnétique Nucléaire (RMN), j'étudie la structure et le fonctionnement de ces GTPases en temps réel dans l'environnement natif des cellules cancéreuses vivantes.

- **Billy Vinette,** PhD Program

  **Supervisor:** Karen Bilodeau, Centre de recherche de l'Hôpital Maisonneuve-Rosemont

  **Project title:** Développement et évaluation d'une intervention éducative numérique visant à soutenir des infirmières dans l'accompagnement à l'autogestion sécuritaire du cannabis

  **Description :** Le but de cette étude est de développer et d'évaluer la faisabilité, l'acceptabilité et les effets préliminaires d'une intervention éducative numérique pour soutenir des infirmières dans l'accompagnement à l'autogestion sécuritaire par le cannabis de jeunes adultes atteints d'un cancer hématologique.

## Characterization of metabolic rewiring and identification of potential therapeutic targets in *CBFA2T3-GLIS2*-dependent AMKL

**Author:** Caroline Capdevielle

**Affiliation:** Laura Hulea and Frederick A. Mallette, Division of Immunology-Oncology, Maisonneuve-Rosemont Hospital Research Centre; Department of Biochemistry and Molecular Medicine, Université de Montréal

**Keywords:** AMKL, CBFA2T3-GLIS2, metabolomics, metabolic vulnerabilities.

**Background information:** The *CBFA2T3-GLIS2 (CG2)* fusion is associated with aggressive (5-year survival less than 20 %) pediatric acute megakaryoblastic leukemia (AMKL; excluding Down syndrome). *CBFA2T3* is normally express in hematopoietic cells and has an important role in stem cells maintenance and self-renewal. In contrast, *GLIS2,* a member of GLI-family transcription factor, is associated with hedgehog pathway activation but is not expressed in normal hematopoietic cells. Several studies demonstrated the importance of metabolic adaptation for leukemic cell survival, revealing vulnerabilities that can be potentially targeted. However, the potential role of *CG2* in metabolic alterations occurring in *CG2*-positive AMKL remains unexplored.

**Purpose of the study:** We propose to investigate how metabolic rewiring associated to *CG2* gene fusions contribute to malignant transformation in order to identify and validate new metabolic therapeutic target.

**Methods:** Using mouse hematopoietic stem and progenitor cells (HSPC) expressing *CG2* gene fusion, metabolic gene expressions were analyzed by RNAseq associated with GC-MS analysis to quantify metabolite levels. In parallel, inhibition of GLIS2 expression/activity by CRISPRcas9 or GANT61, an inhibitor of GLI-family members, were performed in *CG2* positive human AMKL cell line to characterize metabolic changes by Seahorse or GC-MS analysis. To identify therapeutic vulnerabilities in AMKL cell lines, shRNA vectors against *PC, PDK2, GAD1* or AZD7545 pharmacological inhibitor were used in M07e and cell proliferation was measured after 7 days of treatment.

**Results:** Transcriptomic analyses in *CG2*-driven mouse AMKL cells identified numerous genes pertaining to pyruvate metabolism. Such genes included all three subunits of pyruvate dehydrogenase complex

and their regulators (such as Pdk2), but also pyruvate carboxylase (PC). Steady-state metabolite levels analysis by GC-MS showed an increase of pyruvate, aspartate and GABA. Consistently, we observed that GLIS2 inhibition by GANT61 rescued *CG2*-dependent variations in these metabolite levels in *CG2*-positive human AMKL cells. Furthermore, inhibition of GLIS2 by CRISPR-Cas9 alters mitochondrial respiration and glycolysis. Based on transcriptomic analyses, we decided to target *PC* and *PDK2*, to elucidate their functions in AMKL cell line. Using shRNA, we observed a decrease of proliferation 7 days post-infection. In addition, treatment with AZD7545, an inhibitor of PDK activity, has a strong effect on proliferation. Interestingly, we found that GABA, a neurotransmitter, was upregulated after *CG2* or *GLIS2* expression. We generated a shRNA against *GAD1*, an enzyme converting glutamate into GABA to power the TCA cycle during GABA shunting. Downregulation of *GAD1* correlates with decreased proliferation.

**Conclusion:** In conclusion, we propose that *CG2* induces metabolic rewiring of hematopoietic cells to promote leukemogenesis. None of these vulnerabilities have been previously described in AMKL cell lines and represent novel potential therapeutic targets. By using $^{13}C$ stable isotope tracing, we will analyze how metabolic pathways related to pyruvate, aspartate and GABA are dysregulated in *CG2*-dependent cells. All these experiments could lead to improved therapeutic strategies for pediatric AMKL by targeting specific metabolic vulnerabilities.

## The eukaryotic translation initiation factor eIF4E reprogrammes the splicing machinery and drives alternative splicing

**Author:** Mehdi Ghram

**Co-authors:** Gavin Morris, Biljana Culjkovic-Kraljacic, Jean-Clement Mars, Patrick Gendron, Lucy Skrabanek, Maria Victoria Revuelta, Leandro Cerchietti, Monica L Guzman, Katherine LB Borden

**Affiliation:** Katherine Borden's Lab. Institute for Research in Immunology and Cancer. University of Montreal

**Keywords:** Acute Myeloid Leukemia, Eukaryotic Initiation Translation Factor eIF4E, Alternative splicing, Ribavirin

**Backround information:** Aberrant RNA splicing contributes to the pathogenesis of many malignancies including Acute Myeloid Leukemia (AML). While mutation is the best-described mechanism underpinning aberrant splicing, recent studies show that predictions based on mutations alone likely underestimate the extent of this dysregulation.

The eukaryotic translation initiation factor eIF4E, an oncoprotein that has been demonstrated to promote tumorigenesis in different types of cancer, is a post-transcriptional regulator with well-described roles in RNA nuclear export and translation of specific transcripts. Over the past years, our lab has successfully shown a positive response from certain tumor subtypes, including AML, to an eIF4E inhibitor (Ribavirin), *in vitro*, *ex vivo* and in clinical trials

Until recently, nuclear functions of eIF4E have been overlooked, and only mRNAs nuclear export was described. However, over the past few years our lab showed for the first time that nuclear eIF4E is involved in capping, as well as in 3'end processing of subsets of transcripts.

**Purpose of the study:** Given the direct role of eIF4E as a regulator of RNA maturation steps like capping and 3'end processing, we hypothesize that eIF4E is involved in splicing regulation.

**Methods and results:** Using generated stable Osteosarcoma cell lines overexpressing eIF4E, and taking advantage of RNA-seq approaches along with Bioinformatics, we show that elevation of eIF4E reprogrammes splicing of nearly a thousand RNAs in model cell lines. In AML patient specimens, which did not harbour known splice factor mutations, ~4000 transcripts were differentially spliced based on eIF4E levels and this was associated with poor prognosis. Inhibition of eIF4E in cell lines reverted the eIF4E-dependent splice events examined. Splicing targets of eIF4E act in biological processes consistent with its role in malignancy. This altered splicing program likely arose from eIF4E-dependnet increases in the production of many components of the spliceosome including SF3B1 and U2AF1, which are frequently mutated in AML. Notably, eIF4E did not drive mutations of these factors, only their production. eIF4E also physically associated with many splice factors including SF3B1, U2AF1, and UsnRNAs.

**Conclusion:** In all, our studies provide a paradigm for how dysregulation of a single factor can alter splicing and add to the repertoire of oncogenic nuclear functions associated with eIF4E.

## Identification of small molecules for genotype-specific targeted therapies in high-fatality pediatric leukemia

**Author:** Verena Gress

**Affiliation:** Sonia Cellot, Immune Diseases and Cancer Axis, CHU Sainte-Justine Research Center

**Keywords:** AMKL, synthetic mouse models, high-throughput chemical screen, apoptosis, targeted therapies

**Background information:** Acute megakaryoblastic leukemia (AMKL) is a rare high-risk subtype of pediatric acute myeloid leukemia (AML). AML is genetically heterogeneous with recurrent and mutually exclusive oncogenic fusions. Overall, AMKL has a poor prognosis with <40% cure rates due to patients becoming resistant to chemotherapy and suffering from early relapse. Therefore, the development of therapies tailored to the patient situation is urgently needed but greatly limited by the availability of primary sample material. As a result, our research group has established synthetic models of relevant high-fatality pediatric AML with different oncogenic fusions. These models reflect the human disease in a patho-physiological relevant context in mice. Utilizing these models of AML in a high-throughput chemogenomic screen (HTS), we were able to highlight vulnerabilities of pediatric AML towards inhibition of pro-survival proteins of the BCL-2 family.

**Purpose of the study:** This project aims to identify pathways in AMKL that are distinct from other pediatric AML and can be exploited for targeted therapy.

**Methods:** In an initial unbiased HTS, three synthetic models and one patient-derived xenograft (PDX) of different molecular subgroups of AMKL were tested against 12,000 small molecules (including 2,000 FDA-approved compounds). For comparison, other subtypes of pediatric AML (n=19) and normal hematopoietic stem and progenitor cells (HSPC, n=2) were screened alongside. Hits were called when a compound inhibited viability in AMKL cells of more than 75% and at the same time showed less than 30% of cell death in normal HSPCs. Afterwards, dose-response curves of selected molecules were established with determination of the IC50 (half maximal inhibitory concentration). On-target effect of compounds was established by shRNA-mediated knock-down of genes whose proteins are targeted by the drugs.

**Results:** Preliminary results indicate an exploitable vulnerability of AMKL cells towards inhibition of pro-survival proteins of the BCL-2 family. The strongest effect for the induction of mitochondrial apoptosis in our models of AMKL was observed with genetic and pharmacological inhibition of the pro-survival BCL-2 family member BCL-XL. In contrast, inhibition of BCL-2 alone does not induce apoptosis in our models, which is currently the most actively investigated BCL-family member in the clinic (Venetoclax). In addition, one of our selected drug candidates showed promising *in vivo* activity with a significant decrease of infiltration of leukemic cells in bone-marrow and spleen after drug treatment in comparison to vehicle-only controls.

**Conclusion:** In this study we show that high-fatality AMKL are dependent on distinct pro-survival proteins in comparison to other pediatric AML or normal HSPC. Furthermore, this project highlights the

importance of taking advantage of synthetic models of human high-fatality AMKL with relevant oncogenic fusions for the detection of drug candidates. In summary, this project will bring mechanistic insight into the role of apoptotic pathways in AMKL and greatly accelerate the identification of novel options for the treatment of high-fatality infant AML.

## Immunotherapy in acute lymphoblastic leukemia: Investigation of a novel cytotoxicity pathway used by NK cells to kill pre-B leukemic blast

**Author:** Vincent Guitard

**Affiliation:** Élie Haddad, Microbiology, Infectiology and Immunology Department, Université de Montréal; Charles-Bruneau Cancer Center, CHU Sainte-Justine Research Center, Montreal; Department of Pediatrics, Université de Montréal

**Keywords:** leukemia; natural killer; cytotoxicity; immunotherapy; resistance

**Background information:** NK cell-based immunotherapy is a promising therapeutic avenue to prevent precursor-B acute lymphoblastic leukemia (ALL) relapse. Infusions of activated NK cells are safe, well tolerated and do not induce Graft-vs-Host disease. However, ALL are known to be resistant to NK-cell mediated killing. To circumvent this resistance, we have developed an approach based on NK cells obtained *ex vivo* from the NK cell Activation and Expansion System (NKAES). We have found that these expanded NK cells are able to overcome ALL resistance and kill ALL cell lines, after a prolonged contact, via a novel mechanism that has not been reported yet.

**Purpose of the study:** We want to characterize thoroughly this novel NK cell-lytic pathway in order to ultimately harness this mechanism to design new therapies based on the adoptive transfers of expanded NK cells in ALL patients.

**Methods:** We performed degranulation assays and assessed via flow cytometry NK cells activation. We used cytotoxicity assay to evaluate the ability of NK cells obtained from degranulation-defective patients to kill ALL cells. We also investigated the ability of healthy NK cells to kill ALL cells expressing a FADD-dominant negative. We assessed oxidative activity in ALL cells killed by healthy NK cells and evaluated the effects of its moderation by overexpressing proteins known to protect against oxidative stress. Finally, fluorescent labelled inhibitors of caspases probe assays allowed us investigate intracellular activation of specific caspases.

**Results:** We found that a prolonged exposure of NKAES cells to leukemic blast resulted in efficient killing of ALL via a new pathway of cytotoxicity. Indeed, we found that these expanded NK cells do not use the classical degranulation/perforin and/or death receptors cytotoxic pathways to kill ALL cells since (1) degranulation assays did not result in the expression of CD107 on NK cells; (2) expanded NK cells obtained form perforin-deficient patients killed ALL cells; and (3) expanded NK cells killed ALL cells expressing a FADD-DN. We found that expanded NK cell-mediated killing was partially reliant on oxydative damage to the target cell and involved caspases in ALL.

**Conclusion:** Our data challenge the established dogma of ALL resistance to NK cell immunotherapy and open a new therapeutic avenue for refractory ALL patients. The understanding of this new cytotoxic pathway would represent a real breakthrough in the field of cytotoxic cells' biology and cancer immunology. It will allow the design of innovative strategies to enhance the efficacy of NK-cell immunotherapy. Once well understood and mastered, this immunotherapy could be used for the treatment of several other hematologic malignancies.

## CRISPR-based identification of novel cytarabine modulators in acute myeloid leukaemia: deciphering the role of the E3 ubiquitin ligase HERC1.

**Author:** Maja Jankovic

**Affiliation:** Dr. Francois Mercier, Lady Davis Institute (Jewish General Hospital), Department of Medicine (McGill University), Dr. Alexandre Orthwein, Lady Davis Institute (Jewish General Hospital), Department of Oncology (McGill University),

**Keywords:** CRISPR-Cas9 screening, HERC1, E3 Ubiquitin ligases, chemoresistance, acute myeloid leukemia

**Background:** The current standard of care for eligible AML patients is a chemotherapeutic regimen composed of several DNA damaging agents, including the pyrimidine nucleoside analog cytarabine (Ara-C), which target rapidly diving cells. Ara-C is converted to its active triphosphate form and incorporated into the DNA during the S phase of the cell cycle. Problematically, Ara-C containing chemotherapy is effective in curing only ~30% of AML patients, highlighting the need to develop biomarkers that can predict chemotherapy resistance and identify novel drug targets that could improve treatment outcome. Potential targets for pharmacological intervention are E3 ubiquitin ligases, which are part of the ubiquitin-proteasome system (UPS). E3 ubiquitin ligases are highly substrate specific and play major roles in regulating several

aspects of cellular homeostasis. However, the role of E3 ligases in regulating response to Ara-C remains largely unknown.

**Purpose of the study:** The objective of our project is to map the landscape of genes modulating Ara-C response in AML, with a primary focus on delineating the contribution of the E3 ubiquitin ligase HERC1. Our overarching goal is to translate these data into the clinic as biomarkers and/or therapeutic targets.

**Methods:** We performed a genome-wide CRISPR-Cas9 screen in two murine AML cell lines, MLL/AF9 and HoxA9-Meis1. Leukemic cells were transduced *in vitro* with a small guide RNA (sgRNA)-based library targeting 20,611 genes (6 sgRNA/genes). Cells were exposed *in vitro* to Ara-C for a total of 4 days. Pre- and post-treatment, genomic DNA (gDNA) was extracted, amplified by PCR using primers specific to the CRISPR library, and subjected to next-generation sequencing (NGS). The sequencing data was analyzed using two statistical approaches: Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout (MAGeck), and DrugZ, an algorithm specifically developed to identify chemogenetic interactions.

**Results:** Genome-wide CRISPR-mediated screening in two murine AML cells identified the E3 Ubiquitin ligase *HERC1* as common modulator of Ara-C response. CRISPR-based targeting of *HERC1* enhanced sensitivity to both pyrimidine analogs Ara-C and gemcitabine in MLL/AF9 cells. Interestingly, genetic targeting of *HERC1* also sensitized to the purine analogue fludarabine, highlighting a possible class effect. Importantly, we confirmed these findings in the human cell lines U937 and Ramos. Annexin V and cell cycle assays revealed that loss of *HERC1* increased apoptosis through G1/S arrest in MLL/AF9 cells. Finally, gene expression datasets of patient-derived AML samples showed that high expressors of *HERC1* have a poorer survival, providing clinical relevance to therapeutic targeting.

**Conclusion:** Using CRISPR-Cas9 based genetic screening coupled with murine models of AML, we identified *HERC1* as a resistance driver to nucleoside analogues *in vitro* and a predictor of AML patient outcome. *HERC1* has been involved in the modulation of the p53 signaling pathway and our preliminary data suggest this may be an avenue by which *HERC1* impacts Ara-C response. We are currently developing proteomic approaches to characterize the interacting partners of HERC1 in response to Ara-C.

## Improved anti-tumor T cell generation using rapidly differentiated dendritic cells

**Authors:** Annabelle Minguy[1], Jessica Trottier[1], Jaime Sanchez-Dardon[1], Jean-Philippe Bastien[1], Vibhuti Dave[1,2], and Denis Claude Roy[1,3]

**Affiliation:** [1]Division of Hematology-Oncology, Hôpital Maisonneuve-Rosemont Research Center, Université de Montréal, [2]Department of Microbiology, Infectiology and Immunology, Université de Montréal, [3]Department of Medicine, Université de Montréal

**Keywords:** Dendritic cells, graft-versus-leukemia, cell therapy, tumor associated antigen, minor histocompatibility antigen

**Background information:** After allogeneic stem cell transplantation, minor histocompatibility antigens (MiHAs), Wilms-Tumor 1 (WT1) antigen, and other peptides presented by host hematologic cancer (HC) cells can lead to the in vivo expansion of donor T cells crucial for the graft-versus-leukemia effect. While the specific ex vivo production of such leukemia-specific T cells represents an interesting treatment approach, the generation of these antigen-specific donor T cells represents a major challenge.

**Purpose of the study:** We have developed a protocol enabling the infusion of monocyte derived dendritic cell (DC)-induced ex vivo expanded T cells specific for MiHAs, WT1 and other peptides that are preferentially expressed on hematologic cells for the treatment of relapsed HC patients. While it provides interesting results, our 42-day expansion protocol is costly and requires prolonged culture periods during which patient with HC could deteriorate rapidly.

**Methods:** To accelerate T cell production, we compared DCs generated in 3 days (DC3) vs 9 days (DC9). Dendritic cells were generated from monocytes, isolated from peripheral blood mononuclear cells. Immature dendritic cells were treated with the gold standard maturation cocktail, with or without toll-like receptor (TLR) agonist. Dendritic cells were pulsed with WT1 peptide and co-cultured with T lymphocytes for 21 days. WT1-specific T cell reactivity was assessed by tetramer staining, and their functionality by pro-inflammatory cytokines production, degranulation and killing potential.

**Results:** DC3 and DC3-TLR showed small differences in expression of maturation/activation markers and a unique gene signature vs DC9. Importantly, the frequency of WT1-specific CD8 T cells, primed by DC3-TLR, was slightly higher than that in DC9 primed cultures. Upon restimulation with WT1 antigen, significantly more WT1-specific T cells expressed INFγ, TNFα, and CD107a degranulation marker, when primed with DC3-TLR compared to DC3 and DC9. Importantly,

these DC3-TLR-primed WT1-specific T cells were cytotoxic against WT1-expressing autologous blast cells.

**Conclusion:** Together, these data show that matured DC3-TLR have a strong capacity to prime and expand functional anti-tumor specific CD8 T cells and thus offer most interesting features for ex vivo expansion of T cells for cancer immunotherapy.

## Transposable elements have distinct expression profiles in antigen-presenting cells of the thymus

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**Keywords:** transposable elements, thymus, self-tolerance induction, antigen presentation

**Background:** Dysregulation of transposable elements (TE) expression in cancer cells is associated with presentation of TE-derived MHC I-associated peptides, higher tumor infiltration by CD8 T cells and better patient prognosis. This suggests that clonal deletion of TE-specific T cells is incomplete. T cell tolerance is induced in the thymus via presentation of self-antigens by MHC molecules on the surface of thymic antigen-presenting cells (APC). However, little is known about TE expression in thymic APC and so far, reports have failed to reach a consensus as to whether T cells can tolerate TE.

**Purpose of the study**: This study aims to elucidate the implication of transposable elements in the establishment of T cell tolerance.

**Methods:** We decided to analyze TE expression in the thymus using previously published scRNA-seq data since thymic APC are highly heterogeneous cell populations. This dataset comprises 66 human thymic samples ranging from 7 post-conception weeks to 40 years of age. Gene and TE expression were quantified using the kallisto and bustools suite, and data preprocessing was performed with the scatter and scran packages in R.

**Results:** Our analyses showed that the two main populations of thymic APC, dendritic cells (DC) and medullary thymic epithelial cells (mTEC), express larger repertoires of TE than other thymic cells, and at higher levels. However, DC and mTEC exhibit distinct profiles of TE expression: i) >30% of TE subfamilies are differentially expressed between DC and mTEC, and ii) TE subfamilies are typically expressed by a small fraction of mTEC (<15% of cells), whereas TE expression is shared by a larger percentage of DC (>70% of DC). Finally, high TE expression in DC and mTEC is associated with biological processes essential to their APC

function such as antigen presentation by the MHC molecule and interferon signaling.

**Conclusions:** Our study provides a better understanding of the function of transposable elements in the establishment of T cell tolerance. As mTEC and DC express distinct sets of TE, our data suggest that these two cell types have non-redundant roles in the establishment of self-tolerance towards TE sequences. Our work could thus facilitate the identification of TE antigens that could be safely and efficiently targeted for cancer immunotherapies.

## Mouse Models of Pediatric Acute Megakaryoblastic Leukemia Reveal Novel Therapeutic Vulnerabilities

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

**Background information:** The CBFA2T3-GLIS2 fusion oncoprotein is frequently recovered in paediatric non-down syndrome acute megakaryoblastic leukemia (AMKL), and children harbouring this fusion show the worst prognosis compare with other AML. In face of the compelling evidence supporting a role for CBFA2T3-GLIS2 in the etiology of the disease, the molecular mechanisms by which this fusion leads to such refractory leukemia remain to be elucidated.

**Purpose of the study:** We generated a *CBFA2T3-GLIS2*-driven preclinical mouse model of AMKL to *i)* investigate genes/pathways involved in the development of the disease, *ii)* identify novel therapeutic targets leading to growth suppression of CBFA2T3-GLIS2 AMKL cells and *iii*) develop and trial targeted therapies *in vivo*.

**Methods:** We co-transduced hematopoietic stem and progenitor cells (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 syngeneic recipients 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. In addition, we evaluated the effect of a putative inhibitor of the CBFA2T3-GLIS2 fusion in different AMKL cell lines derived from pediatric AMKL patients with the gene fusion (M07e, WSU-AML, RS-1).

**Results:** Transplantation of fetal liver (FL) cells expressing CBFA2T3-GLIS2 induced AMKL in mice. Our model mimics many aspects of human disease, characterized notably by a drastic accumulation of megakaryocytic blasts in the bone marrow. Transcriptomic analysis further showed that FL cells expressing either CBFA2T3-GLIS2 or GLIS2, but not CBFA2T3, display a gene signature that is similar to the expression data from pediatric patients with CBFA2T3-GLIS2. This suggest that modulation of GLIS2 functions might thwart the oncogenic activity of the fusion. Accordingly, we identified a novel inhibitor of GLIS2/CBFA2T3-GLIS2. Most importantly, this inhibitor impaired the transcriptional activity of CBFA2T3-GLIS2 and promoted apoptosis in human AMKL cells.

**Conclusion:** Targeted therapies for the CBFA2T3-GLIS2 subgroup of pediatric AMKL patients are currently inexistant. Our model now serves as a tool to study molecular pathways involved in the disease and allows the identification of genes/pathways that may be targeted by therapeutic drugs. As such, our results support the advance of optimized and targeted therapy specific to this life-threatening subset of AML.

## NR4A3 transcription factor regulates anti-tumor CD8 T cell response

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**Keywords**: CD8 T cells; NR4A3, transcription factor; adoptive cell therapy (ACT)

**Background information**: Adoptive cell therapy (ACT) is a therapeutic anti-cancer treatment that relies on injection of specific anti-tumor CD8 T cells that target and eliminate tumor cells. Unfortunately, this type of treatment is effective only for some patients. ACT ineffectiveness is correlated with poor *in vivo* persistence of T cells as well as their loss of function and exhaustion. Thus, it is important to better understand CD8 T cell differentiation during the anti-tumor immune response to increase the effectiveness of ACT. NR4A3 is a transcription factor that is rapidly induced after CD8 T cell activation. Our group recently showed that NR4A3 deficiency in CD8 T cells promotes memory generation while at the same time enhancing cytokine production. Therefore, we hypothesize that ACT with *Nr4a3*-/- CD8 T cells would provide a better anti-tumor immune response.

**Purpose of the study**: To determine the effect of NR4A3 deletion on ACT effectiveness for the treatment of solid tumors and leukemia in mouse models.

To gain insight into the molecular mechanism of action of NR4A3 by identifying NR4A3 target genes.

**Methods and results**: As a first step, we show that ACT of melanoma-bearing mice with *Nr4a3*-/- effector CD8 T cells provides a better tumor control than with their wild-type counterpart. Furthermore, the therapeutic effect is increased by aPD-L1 therapy. Improved tumor control is associated with an increased proportion of *Nr4a3*-/- CD8 T cells within the tumors and a decrease in their exhaustion and terminal differentiation. scRNA-Seq analysis of tumor-infiltrating lymphocytes further reveals the presence of a distinct progenitor population and an increased proportion of cells enriched for the gene signature associated with aPD-L1 response in absence of NR4A3. Secondly, to identify NR4A3 target genes, we cloned NR4A3 into a retroviral vector and added a molecular tag (3xFLAG) to perform a chromatin immunoprecipitation experiment and sequencing (ChIP-Seq). Then, we have validated the biological activity of the tagged form of NR4A3 by showing its ability to restore the wild-type phenotype in *Nr4a3*-/- CD8 T cells and tested it into a ChIP-qPCR experiment. This established system will be used to identify NR4A3 target genes by ChIP-Seq.

**Conclusion**: NR4A3 deletion in therapeutic CD8 T cells not only enhanced the anti-tumor immune response but also increased the effectiveness of already existing therapy such as immune checkpoint blockade. Modulation of NR4A3 activity represents a promising strategy to generate long-lived and highly functional T cells for ACT.

## Targeting senescence to limit therapy-induced toxicity and improve immune function in pediatric leukemia survivors

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**Keywords:** Senescence, T CD8 exhaustion, secondary infection, chemo/radiotherapy

**Background information:** Chemotherapy and radiotherapy are currently used to cure leukemia in pediatric patients. Despite their efficacy, these treatments increase the risk of developing secondary cancers or persistent infections years after the initial treatment, reducing the long-term survival of patients. Some studies have proven that chemo/radiotherapy induces premature aging of cells through senescence mechanisms. Senescence is beneficial to protect the organism against damaged cell expansion, but the accumulation of senescent cells leads to the development of aging-associated diseases such as cardiovascular diseases and arthritis.

**Purpose of the study:** (1) To evaluate the importance of senescent cells induced by chemo/radiotherapy on immune responses against cancer cells and chronic viruses, (2) with the objective of targeting these senescent cells thanks to senolytics drugs (Navitoclax or ABT-263) to restore potent immune responses.

**Methods and results:** Eight-week-old female C57BL/6J were exposed to X-rays at the single sublethal dose of 6.5 Gy to induce premature senescence phenotype, thus recapitulating what is observed in cancer survivors. Then, to evaluate the impact of senescence on immune function in the context of secondary infection, prematurely-aged mice were infected with the Lymphocytic Choriomeningitis Virus Clone-13 (LCMV Cl-13). Our data showed that the T CD8 lymphocytes presents less proliferation and functional capacities of killing infected-cells during the infection. This mechanism, called T CD8 cell exhaustion, are increasing in mice treated with radiotherapy. Killing of senescence cells thanks to Navitoclax allowed to reduce the T CD8 cell exhaustion and increase the number of effector T CD8 with good functional capacities, allowing a better control of infection by the mice.

**Conclusion:** Our results show us that the killing of senescent cells induced after radio/chemotherapy could be one way to avoid cancer or persistent infections development after leukemia cure for survival patient.

## Cardiovascular responses to acute exercise in survivors of pediatric acute lymphoblastic leukemia

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**Keywords:** Pediatric oncology, Electrocardiogram, QT intervals, Heart rate, Cardiopulmonary exercise test

**Background information:** Anthracycline-related cardiotoxicity is a major cause of mortality and morbidity in pediatric acute lymphoblastic leukemia (ALL) survivors. Electrophysiologic complications and cardiac autonomic dysfunction are both known to be developed by childhood ALL survivors. Standard current methods for detection of cardiotoxicity have limitations, particularly lack of sensitivity for early detection of subclinical cardiac dysfunction. Early detection of cardiac dysfunction remains a cardiologist challenge and is essential to allow optimal therapeutic intervention. Cardiac rhythm problems may be a precursor to symptomatic cardiac disease.

**Purpose of the study:** This study aimed to observe ventricular repolarization and heart rate during a maximal cardiopulmonary exercise test (CPET) in pediatric ALL survivors. We hypothesized that cancer treatments lead to changes in ventricular repolarization that persist over time and that the use of stress allows unmasking electrophysiological abnormalities.

**Methods:** A total of 250 childhood ALL survivors underwent a maximal CPET on ergocycle and their direct oxygen uptake was measured. All survivors were monitored continuously during the test with a 12-lead electrocardiogram. Heart rate, QT interval, and RR interval measurements were performed at rest, at the end of each step, and during recovery. The QT interval was defined as the period from the onset of the Q-wave to the end of the T-wave, measured linearly and was corrected (QTc). Participants were classified into three groups according to their risk level (standard risk (SR), high risk with and without cardioprotectant (HR+DEX and HR)).

**Results:** All survivors (median age: 21 years, 51.5% male) included in the final analysis (n=200) performed a validated maximal CPET. Most participants (n=147) had not reached their predicted maximum heart rate. For the SR group, QTc intervals continued to shorten towards the end of

exercise, unlike the HR and HR+DEX groups (p=0.014). Mean QTc intervals during recovery was higher in the HR group (p=0.029).

**Conclusion:** Cancer and anthracyclines cause persistent changes in heart rate and ventricular repolarization during exercise. These alterations may be an indicator of altered cardiac function. The use of CPET unmasks cardiac rhythm abnormalities in pediatric ALL survivors. It shows the importance of studying the response to exercise to improve early cardiac dysfunction detection.

## Contributing factors to well-being in a sample of long-term survivors of childhood acute lymphoblastic leukemia (cALL): the role of social sharing of emotions

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**Keywords:** emotion regulation, late adverse effects, resilience, social sharing, survivorship

**Background information:** Despite improved 5-year survival rates of 90%, long term cALL survivorship is associated with late adverse effects that negatively affect quality of life. While most of psychosocial research on adjustment to cALL has focused on psychological risk, a persistent result has been that survivors *globally* demonstrate positive adjustment once their treatments are completed. Little is known about why some survivors fare so well their journey with cALL while a minority experience adjustment issues. Risk factors are largely investigated in the childhood cancer survivorship literature. However, protective factors have received little interest so far. Social sharing of emotions, i.e., sharing and processing emotions in a supportive environment, is suggested to promote positive adjustment when facing adversity such as cancer.

**Purpose of the study:** 1) To explore associations between well-being, health status, social support, and emotional regulation strategies 2) To test the social sharing of emotions hypothesis (i.e., interaction between emotion regulation and social support).

**Methods:** We used data from 92 participants from the PETALE cohort (51% female, aged 24 ± 7 years). Measures included well-being (WHO-5), health status (15D), social support (SSQ-6), as well as emotion regulation measures (cognitive reappraisal and expressive suppression (ERQ) and emotional processing and expression (EAC)). We modeled the

odds of high well-being adjusting for health status in logistic regressions and explored the moderating role of social support with bootstrap techniques.

**Results:** Independent of clinical history, high well-being was associated with better health status, higher social support, more frequent use of cognitive reappraisal and emotional processing. We found a main contribution of emotional processing to well-being (OR = 2.12, 95% CI = 1.09-5.37). The interaction between emotional suppression and social support was significant (OR = .40, 95% CI = .13-.79). Probabilities for high well-being were 96% when expressive suppression was low and social support was high.

**Conclusion:** Results suggest sharing one's own emotions may contribute to well-being in long-term cALL survivors. Combining decreasing emotional suppression with promoting supportive social environment could be a promising target for future supportive care interventions in survivors.

## The role of histone H3-K27M mutation in the progression of acute myeloid leukemia

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**Keywords:** Acute myeloid leukemia, H3-K27M, HSCs, Xenotransplantation, Epigenetics

**Background information:** Mutations affecting the epigenetic landscape are frequent in acute myeloid leukemia (AML) and can cause transcriptional reprogramming and influence cell fate. Our lab and others discovered that mutations in histone H3 genes (H3K27M/I), previously found in aggressive childhood glioma, can also occur in AML. These are gain-of-function mutations that inhibit the polycomb repressive complex 2 (PRC2), thus decreasing the repressive H3K27 trimethylation mark and enhancing global gene expression. We observed that these mutations can occur as an early step in leukemogenesis and are present in pre-leukemic cells. We also determined that H3K27M mutation increases the frequency of HSCs, results in a skewed myeloid differentiation, and increases the aggressiveness of AML cells.

**Purpose of the study:** To better understand how the alterations of HSCs unfold following the acquisition of H3-K27M, we sought to investigate

the kinetics of HSC self-renewal and expansion *in vivo* and examine the transcriptional profile of AML patients carrying H3-K27M.

**Methods:** Xenotransplantation: Human CD34+CD38- cells derived from cord blood (CB) were isolated and transduced with either H3-K27M or H3-wt control lentiviral vectors, as done previously. Vectors have a hybrid bidirectional promoter (SFFV/minimalCMV) to drive the expression of both the marker GFP and the insert gene. Four days following transduction, cells were injected intrafemorally into 20 NSG mice per condition. Every 4 weeks, five mice were sacrificed per condition at 4-, 8-, 12- and 16-week time-points. Cells were then extracted from the femurs, tibias and pelvis and grafts were analyzed by flow cytometry. LT- and ST-HSCs, lineage committed progenitors as well as mature lymphoid and myeloid cells were quantified using established CD markers. Bioinformatics: a joint horizontal meta-analysis was performed on TCGA, Leucegene, and beat-AML RNA-seq datasets (969 samples) to identify transcriptional signatures enriched in H3-K27M/I patients (6 samples) and compare it to our in-house scRNA-seq data of H3-K27M vs wt CD34+ cells collected at week 16 post transplantation.

**Results:** Our xenotransplantation data revealed that H3-K27M-driven expansion of LT-HSCs is not a constant, progressive increase but occurs rather rapidly during a short window of time. We observed that the H3-K27M mutation leads to a large expansion between 8-12 weeks, followed by a stable population after 12 weeks. This was in line with our scRNA-seq data showing an increased quiescence signature for H3-K27M HSCs at week 16, compared to wt. On the other hand, our meta-analysis of public RNA-seq datasets of bulk AML revealed that primary H3-K27M samples are enriched in known HSC and quiescence signatures as well as in genes that we found upregulated in the H3-K27M HSC cluster from our scRNA-seq data.

**Conclusion:** Overall, the existence of specific phases of LT-HSC expansion suggests that the H3-K27M mutation drives preleukemic HSC proliferation through a regulated, reversible process. Our transcriptomic data indicate that transcriptional reprogramming granted by H3-K27M during early leukemogenesis are maintained after the progression to full blown disease. This will enable the deciphering of new molecular mechanisms driving the transformation of pre-leukemic HSCs into leukemic stem cells and provide new insights to improve therapeutic options.

## Characterization of cancer peptides cross-presented by dendritic cells

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**Keywords**: Hematologic cancers, therapeutic vaccines, mass spectrometry, dendritic cells, cross-presentation

**Background information**: Leukemia and lymphoma are hematologic cancers affecting both adults and children One promising strategy of treatment is vaccination, which aims to teach the immune system to recognize tumor antigens (TAs) that are present at the surface of tumour cells and not on healthy cells. These TAs, when presented by major histocompatibility complex (MHC) class 1 at the surface of the cells, can be targeted by certain immune cells called CD8+ T cells, which are the ultimate effectors of tumour elimination. Priming CD8 $T^+$ cells requires cross-presentation of TAs by dendritic cells (DCs). Yet, it has been observed that several tumor specific antigens (TSAs) fail to be cross-presented and importantly, many previously described TSAs are generated by the translation of non-canonical transcripts, which often yield short-lived and rapidly degraded proteins. Those are good candidates for direct presentation but may be poor candidates for cross-presentation, although no systems-level proteomic analysis of the repertoire of cross-presented antigens has been reported yet.

**Purpose**: The present project aims to study the origin, characteristics and abundance of peptides which are cross presented by DCs following co-culture with hematologic cancer cells, and to validate their superior immunogenicity.

**Methods:** Stable isotope labeling by amino acids in cell culture (SILAC) allows the incorporation of “heavy” amino acids into the cellular proteome of cells in order to discriminate them from a population of cells grown in normal medium. In this project, dying mouse EL4 lymphoblastic lymphoma cells, which were grown in SILAC medium, are co-cultured with type 1 conventional dendritic cells (cDC1) deriving from mouse bone marrow, which exhibit high cross-presentation capacities. After isolation of the cDC1, their repertoire of MHC-I associated peptides (MAPs), called the immunopeptidome, is analyzed by mass spectrometry, with a focus on SILAC labeled peptides.

**Results**: 20 cross-presented EL4 peptides have been identified, at low levels, in the immunopeptodime of DCs. They all arise from the translation of canonical regions of the genome. This number being too low to draw systemic conclusions, we are currently working on increasing level of cross-presentation and the sensitivity of MAPs detection.

**Conclusion**: In addition to the considerable step forward in tumor immunology, understanding the rules governing cross-presentation may explain why patients respond differently to immunotherapy and will contribute to designing efficient anticancer vaccines.

## The X-linked gene for the helicase DDX3X is required for lymphoid differentiation and MYC-driven lymphomagenesis

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**Keywords:** DDX3, RNA helicase, Germinal-Center, MYC, lymphoma

The X-linked gene *DDX3X* encodes an RNA helicase and is mutated at high frequencies in several types of human B-cell lymphoma. More precisely, *DDX3X* mutations are found in 30% of Burkitt Lymphoma tumors affecting male patients. Females have two active *DDX3X* alleles and males carry a *DDX3Y* homolog on the Y chromosome. Although mutations have been detected in human B-cell lymphoma, the role of DDX3X in B-cell physiological and malignant B-cells is unknown.

The aim of this study is to characterize the impact of *Ddx3x*-depletion in murine hematopoietic cells, more particularly in murine B-cells and lymphomagenesis.

We show here that pan-hematopoietic, homozygous *Ddx3x*-deletion in female mice perturbs erythropoiesis causing early developmental arrest. However, both hemizygous male and heterozygous female embryos develop normally, suggesting that one allele is sufficient for fetal hematopoietic development in females and that the *Ddx3y* allele can compensate for the loss of *Ddx3x* in males. In adult mice, loss of DDX3X affects hematopoietic progenitors, early lymphoid development, marginal zone and germinal center B-cells and lymphomagenesis driven by an E*m-Myc* or l-*Myc* transgene in a sex-dependent manner. Loss of both *Ddx3x* allele abrogates MYC-driven lymphomagenesis in females, while *Ddx3x*-deletion in males does not affect the formation of B-cell lymphoma in both mouse models. Moreover, tumors that appeared in male mice lacking DDX3X showed upregulated expression of DDX3Y indicating a critical requirement for DDX3 activity for lymphomagenesis.

Our data reveal sex-specific roles of DDX3X in erythro- and lymphopoiesis as well as in MYC-driven lymphomagenesis, which are important when considering inhibition of DDX3 as a treatment of B-cell lymphoma.

## Development of NK immunotherapy derived from induced human pluripotent stem cells

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**Keywords:** Immunotherapy; Natural killer lymphocytes (NK); Induced pluripotent stem cells (iPSCs); Chimeric antigen receptors (CAR); Leukemia

**Background information:** Despite of the unprecedented clinical success of autologous CAR-T cell therapy in the treatment of many types of blood cancer, including leukemia, many challenges remain. Major limitations in terms of safety, consistency of efficiency, combined with the complex workforce required to implement those therapies, have raised the need for an "off-the-shelf" cell product, which can be manufactured on a large scale and administered on demand to patients. In this context, development of NK cell-based immunotherapies has emerged as an interesting approach. Indeed, due to their MHC independent lysis activity of malignant cells, allogeneic adoptive transfer of NK cell is safe, with reduced risk to trigger graft versus host reactions. However, biological and technical hurdles related to NK cells gene editing have limited the exploitation of their therapeutic potential.

**Purpose of the study:** We propose to develop an immunotherapy strategy in which CAR-NK cells are derived from iPSC (CAR-iNK). This project aims to evaluate the impact of genomic deletions of negative regulators of the cytotoxic activity of NK cells on both the development of CAR-iNK cells and their antitumor activity.

**Methods:** We will 1) develop iPSC clonal cell lines deficient for targeted regulators, in combination with a CAR expression, by using both CRISPR technology and lentiviral transduction; 2) use modified iPSC cell lines to perform NK lymphocytes *in vitro* differentiation, followed by an amplification step using a co-culture system with artificial antigen-presenting cells genetically modified to express a membrane form of IL-21 ; 3) perform *in vitro* screening studies to assess the antitumor cytotoxic activity of the genetically modified CAR-iNK generated; 4) conduct an *in vivo* preclinical study evaluating the antitumor activity of the most efficient CAR-iNK candidate in a xenogeneic NSG mouse model.

**Results:** The feasibility of CRISPR gene editing in primary NK cells and in iPSCs has been validated. In addition, mature NK cells from 4 different iPSC cell lines have been generated though the optimization of the differentiation protocol. Furthermore, an iPSC cell line expressing a third-generation CAR directed against the CD22 antigen was generated. Screening for the most efficient CRISPR guide targeting selected

negative regulators will be conducted shortly, as the cloning of all sgRNA constructs for CRISPR/Cas9 vector systems is completed.

**Conclusion:** Characteristics of both NK cells and iPSCs have the potential to transform adoptive cell immunotherapy, by enhancing its efficiency and accessibility. The approach of targeting intracellular negative regulators of NK cell cytotoxicity to improve the antitumor potential of CAR-iNK, could provide a proof of concept that producing CAR-NK cells from genetically modified iPSCs is feasible and safe. In addition, study of the loss of function of targeted regulators could be the object of future research and thus, contribute to a better understanding their role in both NK cell development and activation. Moreover, the versatility offered by iPSCs will allow to extend this strategy to other therapeutic targets.

## ThINKK adoptive immunotherapy to prevent acute lymphoblastic leukemia relapse after hematopoietic stem cell transplantation: Mechanisms and biomarkers

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**Keywords:** Acute lymphoblastic leukemia, hematopoietic stem cell transplantation, innate immunity, adoptive immunotherapy

**Background information:** Natural killer (NK) cells are innate lymphocytes able to raise a potent cytotoxic activity against tumor cells. NK-cell-mediated cytotoxicity contributes to early graft-versus-leukemia effect after hematopoietic stem cell transplantation, which is currently the only curative therapy for leukemia relapse. Acute lymphoblastic leukemia (ALL) is the most common childhood cancer affecting lymphoid progenitor cells. ALL cells are known for being resistant to NK-cell-mediated lysis, which could be responsible of early relapse after transplantation and hamper therapeutic use of NK cells. To prevent post-transplant relapse, our laboratory has developed a new therapeutic tool called ThINKK which stimulate the anti-ALL activity of NK cells after transplantation. Although we have demonstrated the efficacy of this innovative approach, the precise mechanisms by which ThINKK-stimulated NK cells kill ALL cells remain unknown.

**Purpose of the study:** The aim of this study is to understand these mechanisms in order to define biomarkers of sensitivity and select the patients whom will benefit from this novel approach.

**Methods:** Here, we used 6 pediatric pre-B ALL cell lines to (1) assess their sensitivity to NK cell mediated killing *in vitro* and in mouse model, (2) assess caspases activation during NK cell mediating killing by flow

cytometry and western blot (3) determine gene expression profiles using next generation sequencing. Finally, the role of the identified biomarker candidates will be investigated either by their depletion via the CRISPR/Cas9 or by their overexpression via lentivirus transfection in these lines.

**Results:** This ongoing study evidence three distinct sensitivity profiles of ALL: (1) ALL highly sensitive to NK cells both, unstimulated and stimulated with ThINKK, (2) ALL highly sensitive to ThINKK-stimulated NK cells but resistant to unstimulated ones and (3) ALL moderately sensitive to ThINKK-stimulated NK cells but resistant to unstimulated ones. ThINKK-stimulated NK cells efficiently lyse ALL cell line harboring a functional TRAIL-R2 pathway (profiles 1 and 2) by caspase dependent apoptosis. ThINKK-stimulated NK cells moderately lyse ALL cell line harboring a deficient TRAIL-R2 pathway (profile 3) by triggering caspase independent cell death. The determination and validation of specific markers associated with these profiles is ongoing.

**Conclusion:** Our results show the efficacy of our innovative approach. ThINKK adoptive immunotherapy does not depend on antigen recognition which reduces the risk of ALL escape. In addition, ThINKK are produced rapidly without any genetic manipulation. This enables early treatment of patients, ensures better safety and reduces the costs of the procedure. The long-term perspective is to offer this innovative immunotherapy to transplanted children with high-risk ALL.

## Building new transcriptomic references from Nanopore data : a benchmarking study

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**Keywords:** Transcriptomics, Nanopore, Pediatric leukemia, Diagnosis

**Background information:** Advances in RNA sequencing (RNAseq) technologies – long reads, in particular – have heralded a new era for RNA biology, where the discovery of new transcript isoforms (such as alternative splicing events or novel long non-coding RNAs) outpaces their integration into reference transcriptomes. The accurate depiction of new transcript isoforms and their inclusion into *ad hoc* reference transcriptomes is important to increase the sensitivity and specificity of transcriptomic analyses. However, to date, there is no 'gold standard' bioinformatics workflow for the robust assembly and discovery of new transcript isoforms from third generation, long-read transcriptomic data.

**Purpose of the study:** Here, we set to agnostically compare and evaluate the predominant de novo assembly algorithms for nanopore RNAseq.

**Methods:** Specifically, we compared Stringtie2, FLAIR, TALON, RATTLE, isONclust and Bambu software packages to evaluate their precision at transcriptome assembly using *Sequins*––synthetic RNA spike-in controls of known composition and abundance. Comparing assemblies obtained with these algorithms and their hyperparameters to the *Sequins* reference assembly provides an empirical estimate of the de novo assembly accuracy, mainly focusing on intron/exon and exon/exon junctions as well as the potential to detect new isoforms.

**Results:** Preliminary results on the popular Minimap2 and Stringtie2 pipeline with default parameters reveal that at least 80% of isoforms are artefactual which translates into a precision and sensitivity of 3% and 10% respectively.

**Conclusion:** This leaves a wide room for improvement which will benefit gene and isoform-level quantification accuracy. Ultimately, we aim to establish an optimized analytical pipeline to facilitate the reliable identification of RNA isoforms in a clinical setting for pediatric leukemia diagnosis.

## New immunotherapy against acute lymphoblastic leukemia using CAR-engineered hematopoietic stem cells under synthetic cell-type specific promoters

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**Keywords** : Hematopoietic Stem cells, Cell-type specific promoters, Chimeric Antigen Receptor, Immunotherapy, Humanized mice

**Background information:** Acute Lymphoblastic Leukemia (ALL) is the most common pediatric cancer and the leading cause of death related to cancer in children. Recently, the use of modified patient cells expressing chimeric antigen receptor (CAR) has emerged as a promising new treatment strategy. CARs are artificial receptors that specifically recognize a surface antigen on target cells and trigger an activation cascade in the genetically-engineered immune cell bearing it in order to destroy the recognized target. As of today, CAR therapy is made by transducing

T cells. However, despite frequent good efficacy of this strategy, some patients do not respond or will relapse after initial response in part due to poor amplification, T-cell exhaustion and/or leukemic cell evasion. Moreover, there is also a potential threat of a serious cytokine release syndrome following rapid expansion and activation of re-injected CAR-T cells.

**Purpose of the study:** To address these issues, we aim to develop an alternative therapeutic approach in which CAR transduction is made in hematopoietic stem cells (HSCs) instead of T cells, allowing a continuous and progressive replenishment of CAR modified cells and so avoiding T cells exhaustion and cytokine release syndrome.

**Methods:** To limit the CAR expression only to target cells, we have designed synthetic T and/or NK cell specific promoters that will constraint the CAR expression to T and/or NK cells progeny. We validated synthetic promoters specificity both *in vitro*, using an artificial organoid thymic differentiation system, and *in vivo* in a humanized mice model. To evaluate both the efficacy and the safety of our therapeutically strategy, we will humanize mice with CAR-CD22-engineered HSC, targeting B cells surface antigen, under the control of the specific promotor. Mice will be challenged with either leukemic cells lines, or patients' leukemic cells to generate preclinical data on the efficacy of this new therapeutically approach that closely mimic the clinical setting.

**Results:** Our results show that we were able to generate new synthetic promoters with a lineage specificity. Moreover, our preliminary data indicate that mice engrafted with GFP-engineered CD34+ cells under synthetic promoters show a similar immune reconstitution of both myeloid and lymphoid cells than control mice. We are currently testing our strategy *in vivo* using HSCs transduce with CAR-CD22 against human B-ALL cell lines.

**Conclusion:** Demonstrating the validity and the functionality of our specific promotor represent not only a major advance in childhood leukemia treatment but also an example of promotor design method that could be applicable to other malignancies and to many others fundamental and applicated research fields. Our new approach could overcome past hurdles by increasing efficacy of CAR immunotherapies while reducing their toxicity.

## Investigating the role of TFEB-mediated autophagy/lysosome biogenesis and mTORC1 pathway activity in the development of B-cell acute lymphoblastic leukemia

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**Keywords:** Transcription factor EB, Acute lymphoblastic leukemia, Autophagy, Lysosome, mTORC1

**Background information:** B-cell acute lymphoblastic leukemia (B-ALL) accounts for approximately 80% of ALL cases. It is both the most common childhood cancer and the most common cause of death among pediatric cancers. While recently improved therapeutic strategies has markedly increased 5-year overall survival rate for pediatric B-ALL, current therapeutic regimens are often associated with severe toxic side effects and relapse. Therefore, much work still needs to be done to understand the complex mechanisms leading to the development and progression of B-ALL and develop more effective drugs based on these mechanisms. Recently, it has been shown that autophagy/endolysosomal system plays an important role in mediating B-ALL development and progression. Additionally, the mTORC1 signalling network has been associated with poor prognosis and chemoresistance of ALL. Transcription factor EB (TFEB), a member of MiT family, is a master regulator for autophagy and lysosome biogenesis and mTORC1 signalling, which may serve as a novel potential target for B-ALL treatment.

**Purpose of the study:** The aim of the study is to determine the role of TFEB-mediated autophagy/lysosome biogenesis and mTORC1 pathway activity in the development of B-ALL and identify a novel potential target for B-ALL treatment.

**Methods:** We firstly generated a B cell specific TFEB knockout mouse model (Tfeb-$KO_{Mb1}$). To determine whether TFEB plays an important role in mediating lysosome activity and function in activated B cells, we used both LysoSensor and lysosome degradation sensor assay. We also checked the expression of lysosomal-associated membrane protein 1 (LAMP-1), a lysosome marker, to investigate the capacity of lysosome in mutant and WT B cells. To investigate the role of TFEB in mediating mTORC1 pathway activity, the expression of phospho-S6 ribosomal (PS6) protein, a key downstream target of mTORC1, was checked. We also investigated the metabolic alterations in mutant and WT B cells by applying protein synthesis assay and glucose uptake assay.

**Results:** We found that the pH of lysosome was higher and the lysosome degradation function was impaired in mutant B cells compared to WT counterpart, suggesting the impaired function of lysosome in mutant B cells. LAMP-1 expression was also decreased in mutant B cells relative to WT B cells, indicating the impaired lysosome biogenesis in mutant B cells. We also found that the expression PS6 was reduced in mutant B cells which suggests the decrease of mTORC1 pathway activity. We further found that ablation of TFEB impaired glucose uptake and ATP synthesis in activated B cells. Interestingly, in the mutant activated B

cells the AID expression was also significantly reduced relative to WT controls. AID is an enzyme that may cause DNA double strain break and mismatch mutation, and tightly correlates with B-ALL progression and poorer prognosis outcomes.

**Conclusion:** Altogether, our results suggest that ablation of TFEB in B cells impairs the development and function of lysosomes and reduces mTOCR1 signalling in activated B cells. These results thus indicates that targeting TFEB in B cells may prevent and cure the development and progression of B-ALL. Further studies including establishing B-ALL humanized mouse models and identifying the role of TFEB in the development of B-ALL on these models will be done.

## Characterization of acute megakaryoblastic leukemia (AMKL) to identify novel cell-surface antigens as potential druggable targets

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**Keywords:** AMKL; pediatric leukemia; surfaceome; biomarkers; therapeutic targets

**Background information:** Acute myeloid leukemia (AML) is a highly heterogeneous disease characterized by large chromosomal rearrangements, such as translocations, inversions or genetic mutations. These genomic alterations affect the normal maturation process of myeloid precursor and lead to their clonal expansion. One of the most lethal subgroup is acute megakaryoblastic leukemia (AMKL), which affects young children mostly under 3 years of age. Recent studies highlighted several gene fusions such as NUP98-KDM5A (N5A) or CBFA2T3-GLIS2 (CG2) that are considered critical transforming events in AMKL. To overcome difficulties associated with studying AMKL, Dr. Cellot's group engineered human models of AMKL by overexpression of recurrent gene fusions in cord blood hematopoietic stem and progenitor cells (CB-HSPCs). These cells serially engraft in immunodeficient mice to generate AMKL with expression profiles similar to human patient samples. This system thus represents an exquisite model to understand the biology of AMKL.

**Purpose of the study:** The goal of this study is to characterize AMKL specimens to identify surface proteins as potential biomarkers or therapeutic targets.

**Methods:** To identify proteins expressed at the surface of AMKL cells, we used the cell surface proteomic approach developed in Dr. Roux's

group and generated datasets representative or NUP98 rearranged (NUP98r) and CG2 AMKL subsets. In this project, we used two independent synthetic CG2 AMKL models (CG2-I732 and CG2-I749), along with previously reported N5A synthetic and NUP98-BPTF (NTF) patient-derived-xenograft (PDX) AMKL models. CHRF288-11 and M07e cell lines expressing N5A and CG2 fusion respectively were also included. To highlight AMKL-specific surface proteins, we also analyzed the surfaceome of normal CB-HSP expanded in culture. To compare these datasets we used bioinformatics tools developed in our lab and generated cell surface maps specific for NUP98r and CG2 AMKL but not expressed on their normal counterpart. Finally, we validated the presence of the most pertinent cell surface antigens using flow cytometry.

**Results:** Our approach generated proteomic datasets highly enriched in cell surface proteins for the AMKL samples. To highlight a specific cell surface maps for NUP98r and CG2 subgroups we chose to combine CHRF288-11, N5A and the NTF PDX model as the NUP98r signature and M07e, CG2-I732 and CG2-I749 as a CG2 signature. We then compared these signatures with the CB-HSPC surfaceome, which allowed us to determine an AMKL-specific signature with potential biomarkers and druggable targets not present in normal cells. Among the 460 cell surface proteins detected, we identified novel genotype-specific AMKL biomarkers and key cell membrane receptors of mTOR and JAK-STAT signaling pathways. We selected a subset of commercially available antibodies targeting cell surface proteins related to these signaling pathways and validated their specificity at the cell surface of AMKL subgroups using flow cytometry.

**Conclusion:** This project combines two complementary expertise in order to better characterize AMKL subgroups and highlight cell-surface antigens with high therapeutic interest. We highlighted cell surface maps specific for NUP98r and CBFA2T3-GLIS2 AMKL subgroups. From these datasets, we will be able to generate a list of potential biomarkers and therapeutic targets focusing primarily on mTOR and JAK/STAT related cell surface antigens. To continue our study, we will select the top five most pertinent cell surface antigens and assess their roles *in vitro* using loss-of-functions approaches. Our ultimate goal will be to develop new therapies targeting AMKL and improve the care of pediatric patients.

## Single Cell Magneto-Optical Capture (scMOCa): Developing a new method for antigen-specific T cell isolation

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**Keywords:** T cell therapy, antigen specific T cells, TCR discovery, machine learning, calcium flux

**Background information:** T cell therapies are revolutionizing the treatment of pediatric leukemia. Many emerging adoptive T cell therapies rely on the transduction of T cells with a predetermined antigen receptor to re-direct their specificity towards tumor-specific antigens. The identification of these T cell receptor (TCR) sequences generally relies on the isolation of antigen-specific T cells and subsequent sequencing of their TCRs. Despite the development of multiple platforms for TCR discovery, this process remains time consuming and/or biased in the quality of TCR identified, representing a critical bottleneck for broader application of TCR-engineered cell therapies.

**Purpose of the study:** Here, we propose to adapt image analysis and machine learning tools as well as biophysics-based methods to develop a novel approach for the identification and isolation of antigen-specific T cells expediting tumor antigen-specific TCR discovery.

**Methods:** Combining time-lapse calcium ($Ca^{2+}$)-imaging, machine learning algorithms, and single cell Magneto-Optical Capture (scMOCa), we are developing $Ca^{2+}$-scMOCa for the isolation of antigen-specific T cells. Briefly, T cells are labeled with a $Ca^{2+}$ indicator dye and cultured with peptide-pulsed antigen presenting cells. Machine learning algorithms trained on the $Ca^{2+}$ flux patterns of antigen-specific T cells automatically detect the cells of interest among a polyclonal population. The algorithm will then direct laser illumination to antigen-specific T cells to tag these cells with biotin crosslinked to the plasma membrane via a method known as Cell Labeling via Photobleaching (CLaP)). Streptavidin-conjugated magnetic beads and custom magnetic isolation techniques allow for the isolation of rare cells with high purity.

**Results:** We have generated an *in vitro* model that allows for the efficient and robust activation of antigen specific T cells. We developed programs to automatically segment (identify), track (through space and time) and measure the fluorescence of labeled $CD8^+$ T cells from time-lapse imaging datasets and used the data to train and validate a machine-learning algorithm model for the automated identification of

antigen-specific T cells. In parallel, we are optimizing parameters for the biotin tagging of antigen-specific cells and their magnetic isolation with high purity.

**Conclusion:** This study represents a proof-of-concept for the isolation of antigen specific T cells using TCR transgenic mouse models. This approach will be adapted to more complex scenarios and clinical applications (e.g. identification and purification rare antigen-specific T cells of different affinities from human peripheral blood mononuclear cells). Once optimized, this technology could help fast-track TCR sequence discovery in the context of personalized medicine pipelines.

## Feasibility, acceptability, and preliminary efficacy of the Lymfit intervention to foster motivation towards physical activity among young adult lymphoma survivors: A pilot randomized controlled trial

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**Key words:** Pilot randomized controlled trial; young adult lymphoma survivors; exercise motivation; self-determination theory

**Background**: Lymphoma and its subtypes are among the most commonly diagnosed cancers in young adults (YA) aged 18 to 39. Most types of lymphoma are often treated with curative therapies. Unfortunately, treatment side effects, and unhealthy or sedentary lifestyles can substantially affect the quality of life and long-term cancer prognosis outcomes among YA cancer survivors.

A growing body of evidence reveals significant associations between physical activity (PA) and cancer-related outcomes. Evidence suggests that specific frequency, intensity, timing, and types of PA can have positive effects on cancer treatment processes and outcomes. PA is also significantly associated with enhanced health-related quality of life among cancer survivors. Despite sustained efforts in the development and implementation of exercise interventions geared towards specific cancer populations and age groups, evidence suggests that lymphoma survivors' exercise engagements remain suboptimal. This warrants the development of an intervention that is theoretically guided, and specific to cancer type and age, to enhance PA levels sufficient to improve cancer outcomes. Grounded in self-determination theory, the Lymfit intervention (an individualized exercise program with bi-weekly

kinesiologist support -and an activity tracker) can foster autonomous motivation toward PA.

**Purpose**: The purpose of this two-phase study is to evaluate the feasibility, acceptability, and preliminary effects of Lymfit on YA lymphoma survivors' motivation to engage in PA.

**Methods:** In the phase I proof-of-concept study, the research team tested the technological and logistical challenges of the intervention. In the phase II pilot randomized controlled trial (RCT), 28 YA lymphoma survivors will be randomly allocated to receive either the 12-week Lymfit intervention or be on a wait-list. Feasibility and acceptability of Lymfit will be assessed using predetermined feasibility progression criteria and an acceptability questionnaire. Preliminary efficacy will be assessed using self-report questionnaires at baseline and post-intervention on four study outcomes: psychological need satisfaction, exercise motivation, PA level, and quality of life.

**Results:** Twenty YA lymphoma survivors participated as patient partners in phase I. Eighteen participants had completed the intervention and the post-intervention follow-up. The results demonstrated the research team's capability to deliver the exercise program virtually and to capture Fitbit data through a secured server. Recruitment of the phase II pilot RCT is currently underway.

**Implications:** The results of this study are expected to help in mapping out the optimal design of an effective exercise program for improving cancer outcomes and quality of life in YA lymphoma survivors. If Lymfit shows desired effects, it can offer an innovative care option to enhance the quality of post-treatment YA cancer survivorship care.

## p53-Dependent Induction of P2X7 on Hematopoietic Stem and Progenitor Cells Regulates Hematopoietic Response to Genotoxic Stress

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**Keywords:** Hematopoietic Stem Cells, P2X7, p53, Genotoxic Stress, Leukemia

**Background:** Hematopoietic stem cells (HSCs) are the precursors of all blood cells. Cytotoxic damage in HSCs in cancer patients undergoing chemotherapy or radiotherapy results in a long-term predisposition to bone marrow failure and hematologic malignancies. Transcription factor p53 is the major mediator of cellular response to stress. We recently identified 39 novel p53-regulated genes within our HSC RNA-seq and

p53 ChIP-seq datasets, and discovered the p53-dependent induction of P2X7, a major receptor for extracellular ATP, in HSCs in response to irradiation (IR). In the work recently published in *Cell Death & Disease*, I demonstrated the role of P2X7 in HSC response to acute genotoxic stress, with P2X7-loss significantly extending mouse survival in IR-induced hematopoietic failure. I also further demonstrated the role of P2X7 in the loss of HSC regenerative fitness following sublethal irradiation. However, the molecular mechanisms of P2X7 in HSC stress response are poorly understood and the efficacy of targeting P2X7 in irradiation induced bone marrow failure has not been tested.

**Objective:** I have confirmed that P2X7 expression is induced on HSCs in response to genotoxic stress via p53-dependent mechanisms and that P2X7 signaling plays a key role in the loss of HSC fitness. My current aims are (i) to explore the molecular mechanisms through which P2X7 modulates HSC response to genotoxic stress; (ii) to validate P2X7 as a drug target for pharmacological modulation of HSC fitness and hematopoietic response to genotoxic injury.

**Methods and results:**

***I) P2X7-agonist ATP in HSC genotoxic stress: (a)*** Extracellular ATP will be measured in plasma and bone marrow aspirates, comparing control and irradiated mice of WT, *P2X7*$^{-/-}$ and *p53*$^{-/-}$ genotypes, using a dedicated ATP ELISA. ***(b)*** The effect of ATP on HSC function will be studied *ex vivo* in cultures of murine HSCs from WT and *P2X7*$^{-/-}$ mice. HSC responses will be compared between IR-treated and control cells, with/without ATP-stimulation, across a range of doses and time-points, assessing cell viability, cell cycle state, and DNA damage levels. Cell proliferation and clonogenic potential will be assessed with CFU assays.

***II) P2X7 in the in vivo response to IR:*** I demonstrated extended survival of *P2X7*$^{-/-}$ relative to WT mice following IR, showing their enhanced resistance to IR-induced hematopoietic failure. ***(a)*** The underlying mechanisms are now explored by tracking HSC and blood cell numbers in WT and *P2X7*$^{-/-}$ mice across a time-course after IR. ***(b)*** Mouse-to-mouse BM transplantations have been set up to examine the relative role P2X7 on HSCs, other hematopoietic cells, and the niche in the response to IR. ***(c)*** I will further test whether pharmacological blockage of P2X7 *in vivo*, with oATP or AZD9056 (antagonist), can similarly extend mouse survival and promote resistance to IR-induced hematopoietic failure.

**Conclusion:** Our work will provide novel insights into the mechanisms of HSC response to genotoxic stress, and test P2X7 as a possible drug target for modulating the side-effects and toxicities of chemotherapy and radiotherapy on the hematopoietic system of cancer patients.