Cole Foundation

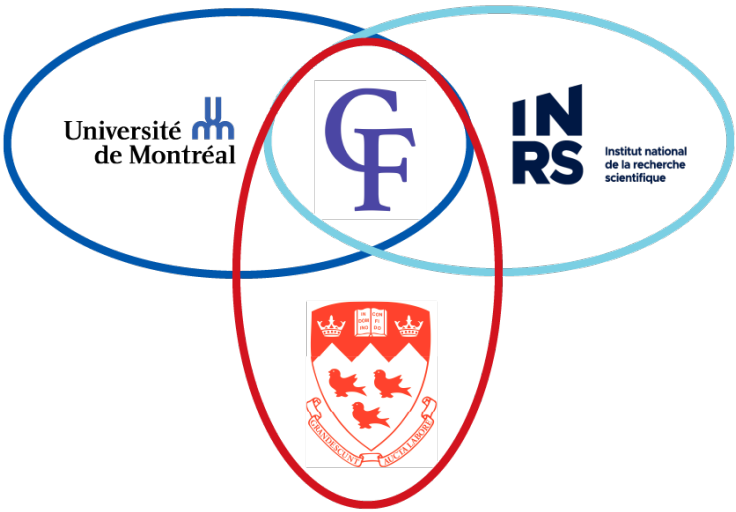
«Research Celebration Day»

17th Year

Journée «Célébrons la recherche»

de la Fondation Cole

17e 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 2023 Fellows, the Fellowship programme has supported more than 220 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 $13 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 childcare 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 childcare 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 K. Kaplan** – Board Member
- **Mr. David Laidley** – Board Member
- **Ms. Anne Lewis** – Board Member
- **Dr. Evan Lewis** – Board Member
- **Mr. Bruce McNiven** – Board Member
- **Ms. Emma Tibaldo** – 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 2023, le programme a appuyé plus de 220 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 13 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 santés'intéressant aux soins et à la recherche en pédiatrie et à la découverte d'un traitement curatif pour la leucémie. Elle a été créée à la mémoire de sa fille, Penny Cole, emportée par la leucémie plus de dix ans avant. La Fondation appuie aussi des causes communautaires louables. Grâce à la Fondation, Jack Cole a donné des millions de dollars à des services de santé de Montréal. Parmi ses grandes réalisations figurent son soutien du Laboratoire Penny Cole à l'Hôpital de Montréal pour enfants et la création de la chaire Jack Cole en oncologie-hématologie pédiatrique à l'Université McGill.

Depuis le décès de son maître d'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 K. 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
- **Mme Emma Tibaldo** – Membre du conseil d'administration

Friday, April 28th, 2023

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 Karine Bilodeau** – Research Centre of the Maisonneuve-Rosemont Hospital
  Co-construction de parcours d'apprentissage avec et pour les jeunes touchés pas un cancer hématologique : le projet PAC SAC
- **Dr Morgan Craig** – Research Centre of the Sainte-Justine University Hospital
  Mathematical modelling to evaluate clonal reduction strategies to treat AML
- **Dr Caroline Lamarche** – Research Centre of the Maisonneuve-Rosemont Hospital
  Treg exhaustion and its implication for cell therapy

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

Keynote speaker: Dr Jonathan Bramson

Adventures in Cell Therapy

3:15 pm

Reception

Dr Jonathan Bramson, PhD, is the Vice Dean, Research for the Faculty of Health Sciences and a Professor in the Department of Medicine at McMaster University. He holds the John Bienenstock Chair in Molecular Medicine. His research is focused on the use of cells as drugs. Specifically, his lab is developing methods to manufacture white blood cell "drugs" that attack cancer. To optimize the anti-tumor activity of these white blood cell drugs, his research team is using a combination of genetic engineering, synthetic biology and chemical biology approaches to re-wire relevant signaling pathways within white blood cells to bolster their anti-tumour potency.

THE 2023 – 2025 COLE FOUNDATION FELLOWS

McGill University

■ **Madelyn Abraham,** PhD program

Supervisor: Sonia Del Rincon, Experimental Medicine, Lady Davis Institute and Koren Mann, Experimental Medicine, Lady Davis Institute

Project Title: Understanding STAT6-D419N-mediated microenvironmental remodeling in relapsed and refractory DLBCL.

Description: Our lab has shown that mutations in STAT6 are enriched in tumour cells in patients with relapsed Diffuse Large B Cell Lymphoma. Interestingly, we have also found that when a STAT6 mutation is present, tumours have increased invasion of CD4+ T cells. My ongoing research is aiming to spatially immunophenotype DLBCL tumours, to better understand how STAT6 mutations can induce microenvironmental remodelling and therapeutic resistance.

■ **Dania Shaban,** PhD program

Supervisor: Anastasia Nijnik, Department of Physiology, McGill University

Project title: Characterizing the effects of the loss of MYSM1 catalytic activity on tumor cell physiology and antitumor immunity.

Description: MYC is overexpressed in more than 70% of cancers. In child and young-adult patients with non-Hodgkin B cell lymphoma MYC overexpression is strongly linked to poor prognosis. We demonstrated that protein MYSM1 cooperates with MYC to regulate gene expression. In the current project we will therefore explore MYSM1 as a putative drug-target, using murine models of B cell lymphoma and studying its roles in the tumor cells and in antitumor immunity.

Université de Montréal

■ **Capucine Bourel,** PhD program

Supervisor: Sylvie Lesage, Cellular Immunogenetics, Centre de Recherche de l'Hôpital Maisonneuve-Rosemont

Title: Characterization of human HLA-DR+NK cells

Summary: Nous avons préalablement identifié une population de cellules NK ayant de fortes propriétés antitumorales chez la souris. Notre projet vise à identifier et caractériser l'homologue humain de cette population de cellules. La caractérisation de ces cellules pourrait, à terme, permettre l'optimisation d'immunothérapies utilisant les cellules NK pour traiter les leucémies.

- **Fanny-Mei Cloarec-Ung,** PhD program

  Supervisor: David Knapp, Cellular Engineering, IRIC
  Project title: Optimisation of a powerful model system to study RUNX1 R204Q mutation implicated in pediatric leukemia.

  Description: In this project, we are combining our optimized genome engineering approach together with cutting edge 3D model systems to model the RUNX1 mutations in the context of leukemia. This will allow us to study the precise cellular mechanisms of these mutations and their therapeutic responses.

- **Sarah Denford,** PhD program

  Supervisor: Brian Wilhelm, IRIC, Medicine

  Project title: Characterization of biomarkers in KMT2A-translocation acute myeloid leukemia

  Description: This project aims to characterize protein biomarkers previously identified in acute myeloid leukemias involving KMT2A-translocations. In a series of in vitro experiments, we are identifying the regulation, activity, and downstream effects of the expression of proteins putatively involved in KMT2A-fusion leukemogenesis.

- **Victor Gife,** PhD program

  Supervisor : Laura Hulea, Centre de Recherche de l'Hôpital Maisonneuve-Rosemont, François Mercier, Institut Lady Davis

  Titre du projet : Caractérisation du rôle de eIF4A dans la résistance des cellules de leucémie myéloïde aiguë à la chimiothérapie et aux thérapies ciblées.

  Description : Ce projet se concentre sur les adaptations métaboliques des cellules leucémiques face aux traitements anti-leucémiques actuels. L'inhibition du facteur de traduction eIF4A, par une petite molécule de la classe des Rocaglates - CR-1-31-B, est une cible intéressante pour créer des vulnérabilités métaboliques et prévenir le développement de la résistance aux traitements.

- **Leila Jafarzadeh,** Post PhD program

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

  Project Title: Multiplex cellular engineering of CAR T cells to Improve Anti-Leukemia Effect and Persistence.

  Description: My project will be to produce CAR T cells which will be engineered to be resistant to the effect of suppressive receptors and pathways. My aims are: 1-To assess the potential synergy of targeted CD5 with other inhibitory co-receptor loss-of-function to promote

chimeric antigen receptor (CAR) T cell anti-tumor function against leukemia/lymphoma cells and 2-To perform a targeted CRISPR-Cas9 screen to identify novel regulators/co-regulators of CAR T cell dysfunction in the treatment of hematological malignancies using our T-cell exhaustion transcriptome dataset.

- **Nitya Khetarpal,** PhD program

  Supervisor: Katherine Borden, Structure and Function of the Cell Nucleus Research Unit, IRIC

  Project title: Investigating novel roles of eIF4E in splicing in leukemia

  Description: The cancer-driving protein eIF4E rewires splicing, a process that regulates most human RNAs. We aim to uncover the unique regulatory RNA sequence and/or structural elements required for eIF4E-dependent splicing events to occur, and better understand their impacts in high-eIF4E cancers, with a focus on acute myeloid leukemia.

- **Alice Mac Donald,** PhD program

  Supervisor: Elie Haddad, CHU Ste Justine Research Centre

  Project title: Développement de nouvelles immunothérapies CAR-NK basées sur la modification génétique de cellules souches pluripotentes induites

  Description: This research project addresses the challenges associated with adoptive cell transfer therapy for blood cancer treatment. We aim to develop CAR-NK cells derived from induced pluripotent stem cells (iPSCs) deficient in negative regulators of NK cell cytotoxicity, which can provide a standardized and abundant source of therapeutic cells with enhanced anti-tumor activity.

- **Eric Gyres Sonounameto,** PhD program

  Supervisor: Nathalie Labrecque, Centre de recherche de l'Hôpital Maisonneuve-Rosemont

  Project title: Caractérisation du mécanisme moléculaire d'action de NR4A3 lors de la réponse des lymphocytes T CD8+

  Description: Mon projet permettra d'élucider les mécanismes moléculaires par lesquels le facteur de transcription NR4A3 influence la différenciation des LT CD8+ afin d'identifier ses gènes cibles, et d'évaluer l'intérêt d'en faire une cible thérapeutique dans le traitement des leucémies.

- **Clara Soulard,** PhD program

  Supervisor: Elie Haddad, CHU Sainte-Justine Research Centre

  Project title: Innovative immunotherapy based on the transplantation of genetically modified hematopoietic stem cells for the expression of chimeric antigen receptors in T cell progeny.

  Description: Although CAR therapies are promising for the treatment of acute lymphoblastic leukemia, some patients do not respond or will relapse. We aim to develop an alternative therapeutic approach in which CAR transduction is made in hematopoietic stem cells allowing a continuous and progressive replenishment of CAR modified cells.

- **Rui Zhang,** Post-PhD program

  Supervisor : Vincent-Philippe Lavallée, CHU Sainte-Justine Research Centre

  Project title: Characterization of the mast cell progenitors in primary t(8;21) AML

  Description: Acute myeloid leukemia (AML) is a highly heterogeneous disease characterized by different genetic and molecular abnormalities, which plays a major role in both treatment and prognosis. We will use single cell RNA sequencing to identify and characterize a mast cell population which is abundant in a subtype of AML.

Title: Developing a CAR-NK cell immunotherapy against severe SARS-CoV-2 for immunocompromised pediatric HSCT recipients

Author: Mila Bjelica

Co-Authors: Hugo Roméro, Tram NQ Pham, Kathie Béland, Anne Duchesne, Marilou Henri,Véronique Lisi, Vincent-Philippe Lavallée, Martin Smith, Étienne Gagnon, Andrés Finzi, Éric A Cohen, Élie Haddad

Affiliation: Dr Elie Haddad, Pediatrics, CHU Sainte-Justine and Dr Etienne Gagnon, Microbiology, Infectiology and Immunology, University of Montreal

Key words: Immunotherapy, infectious disease, NK cells, post-HSCT treatment, chimeric antigen receptor (CAR)

Background: Hematopoietic stem cell transplants (HSCT) remain the leading therapy for highrisk/ relapsed pediatric leukemia. Unfortunately, this therapy leaves children at a high risk of severe infectious disease by pathogens that are otherwise relatively limited in healthy peopleone of such is SARS-CoV-2, with a 6% mortality rate in children having received a HSCT. NK cells are critical in controlling SARS-CoV-2 infection. Unlike with T cells, adoptive NK cell transfer shows a lower risk of Graft versus Host Disease, has an innate ability to destroy virally infected cells, and does not cause a cytokine storm – all qualities which are critical for treating infectious disease. Importantly, NK cell therapies can be produced for off-the-shelf use, to be available as soon as they are needed, since NK transfer does not require a matching donor. As such, we propose to develop chimeric antigen receptor (CAR) NK cells specific against SARSCoV- 2 to combat severe COVID19 in these patients.

Purpose: We aim to produce anti-SARS-CoV-2 CAR-NKs and to assess their efficacy and specificity in cell and mouse models.

Methods: NK-optimized CAR constructs were created by deriving ScFV sequences from an antibody isolated from a patient with COVID19 and adding them to KIRS2/DAP12 signalling domains. NK cells were expanded from the peripheral blood of healthy adults using K562 mbIL-21 feeders, depleted of CD3+ cells and transduced with a baboon envelop pseudotyped lentivirus vector coding for our CAR constructs. The efficacy of the CAR-NKs was tested by cytotoxic assays against 697 expressing spike and CALU-3 infected with SARS-CoV-2.

Results: CAR-NK cells showed significantly more killing of targets expressing spike, compared to targets not expressing spike: 34.17%-46.37% increase in specific lysis, depending on E:T ratio ($p<0.001$, n=3). CAR-NKs also showed improved cytotoxicity compared to untransduced NK cells: 37.62%-52.92% increase in specific lysis, depending on E:T ratio ($p<0.001$, n=3). Early data indicates that CARs are also effective and specific when tested against SARS-CoV-2 infected CALU-3 cells.

Conclusion: Preliminary data suggests our anti-spike CAR-NK cells against SARS-CoV-2 are target specific and have a more potent antiviral effect than untransduced NK cells. With these encouraging results, we are also currently developing CAR-NK cells against cytomegalovirus, an opportunistic pathogen accountable for major complications post-HSCT. Ultimately, we hope to develop off-the-shelf immunotherapies against viral diseases that cause significant complications in children treated for blood cancers, which are effective with minimal toxic side effects.

Title: Exercise prescription based on a Six-Minute Walk Test in survivors of childhood acute lymphoblastic leukemia

Author: Émilie Bertrand

Affiliation: Daniel Curnier and Vincent Jacquemet, CHU Sainte-Justine Research Center

Keywords: Pediatric oncology, Individualized training intensities, Ventilatory threshold, Six-Minute Walk Test, Cardiopulmonary exercise test

Background information: Exercise is beneficial for cancer patients and survivors. Individualized training intensities should be prescribed according to variables determined from a maximal cardiopulmonary exercise test. However, access to this technology is limited. The Six-Minute Walk Test (6MWT) is a valid and safe field test for assessing aerobic capacity in childhood acute lymphoblastic leukemia (ALL) survivors.

Purpose of the study: The first aim of this study was to propose a specific 6MWT equation to predict ventilatory threshold in childhood ALL survivors. The second objective was to compare measured heart rate (HR) at ventilatory threshold with recommended exercise intensity levels for cancer patients and survivors.

Methods: Childhood ALL survivors (n=154) completed a 6MWT and a maximal cardiopulmonary exercise test with gas exchange analysis. Participants were randomized into 2 groups to predict the ventilatory threshold equation (n=107) and to validate it (n=47). Backward linear regression analyses were used to determine the prediction equation. The root mean square error (RMSE) was used to measure the accuracy

of the predicted HR at ventilatory threshold on the validation group. Measured HR at ventilatory threshold were compared to moderate intensity (40–59% HR reserve (HRR)) and vigorous intensity (60–89% HRR).

Results: The equation was [HR ventilatory threshold = (0.074 x age) + (0.218 x HR end 6MWT) + (0.016 x cumulative doxorubicin dose) – (0.051 x height) – (0.835 x years since the end of treatment) – (0.115 x physical activity level) + (0.010 x distance 6MWT) + (0.142 x HR rest) – (0.492 x rating of perceived exertion) + 126.79] (p=0.001, R2=0.271). The resulting RMSE was 14.5 bpm. Four participants had their ventilatory threshold below 40% HRR, 37 between 40–59% HRR, 100 between 60–89% HRR, and 11 above 89% HRR (median HR at ventilatory threshold 70.8% HRR; range 16.4–118.9% HRR).

Conclusion: These results reinforce the utility of assessing the functional capacity of patients with a 6MWT to propose an individualized training program without maximal exercise test. The high heterogeneity in ventilatory thresholds by %HRR may explain the different training responses by %HRR. A training intensity based on a percentage of ventilatory threshold would be appropriate if maximal exercise test is not available for childhood ALL survivors.

Title: Uncovering the complex role of social support in long-term adjustment to childhood acute lymphoblastic leukemia (cALL): an interpretative phenomenological analysis of experiences of young adult survivors

Author : Camille Bourdeau

Co-authors: Andy Charbonneau; Carole Provost, RN; Émélie Rondeau, MSc; Caroline Laverdière, MD, Roxane de la Sablonnière, PhD, & Serge Sultan, PhD

Affiliation: Serge Sultan, PhD, Department of Pediatrics, Université de Montréal; Research Centre, Sainte-Justine University Health Centre

Keywords: Childhood acute lymphoblastic leukemia (cALL), long-term survivorship, social support, interpretative phenomenological analysis, psychological adjustment

Background information: Long-term survival of childhood leukemia is associated with psychosocial difficulties affecting quality of life in adolescent and young adults. Social support is seen as key to long-term adjustment in this population. Although forms of beneficial social support have been identified, little information is available as to the point of view of survivors and potential mechanisms of action.

Purpose of the study: To identify and understand the explanatory mechanisms of social support in the adjustment of long-term survivors of childhood acute lymphoblastic leukemia (cALL) by focusing on their subjective experience.

Methods: Long-term cALL survivors (expected n=12, 10 women, age=19-27) were recruited in the long-term follow-up clinic of Sainte-Justine UHC. We led in-depth individual narrative interviews (McAdams, 2007) and analysed them with an interpretive phenomenological analysis (Smith et al., 2022). The elements of each participant's discourse related to social support were grouped into personal experiential themes. A post-hoc quantitative description of well-being (WHO-5), social support (SSQ-6), and health status (15D) is also available.

Results: Recruitment and analysis are in progress (N=8 interviews to date). The verbal material highlights the meaning that participants give to their cALL experience and the role of social support in their psychological adjustment. The preliminary themes suggest that unconditional family support, normative interactions with others, support towards independence, and support in discovering new areas of interest appropriate to their physical condition are major explanatory factors of beneficial social support. Preliminary unfavorable forms of social relationships include minimizing the impact of cALL on well-being, unsolicited pity behaviors, and a perception that their entourage does not have the capacity to respond adequately to survivors' distress.

Conclusion: Focusing on the subjective experience of support of long-term cALL survivors provides a deep understanding of social support mechanisms. These may help to conceptualize impactful supportive attitudes. The results may translate to counseling or psychoeducation strategies focused on caretakers early in the trajectory. This will help meet the unique needs of adolescent and young adult long-term cALL survivors.

Title: 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, interactome

Background information: Acute megakaryoblastic leukemia (AMKL) represents ~10% of pediatric acute myeloid leukemias and is associated with extremely poor prognosis. Importantly, the *CBFA2T3-GLIS2* (CG2) gene fusion is the most observed lesion amongst all pediatric AMKL patients (~20%), is associated with chemotherapy resistance and shows the poorest prognosis (<20%; 5-year survival) compared to the other gene fusions. *CBFA2T3* is normally expressed 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 not expressed in normal hematopoietic cells. Several studies demonstrated the importance of metabolic adaptation for the survival of acute myeloid leukemia cells, but also the therapeutic potential of targeting metabolic vulnerabilities. However, the potential role of *CG2* fusion in causing metabolic alterations in 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 (HSPCs) expressing *CG2* gene fusion, metabolic gene expressions were analyzed by RNA-seq associated with GC-MS analysis to quantify metabolite levels. In parallel, inhibition of GLIS2 expression/activity by CRISPRcas9 was performed in *CG2*-positive human AMKL cell line to characterize metabolic changes by RNA-seq, Seahorse or GC-MS analysis. To identify therapeutic vulnerabilities in AMKL cell lines, shRNA vectors against *PC, PDK2* or the pharmacological inhibitor AZD7545 were used, and cell proliferation was measured after 7 days of treatment. Finally, to identify the partners of *CG2* fusion proteins and their role in AMKL, we performed a protein proximity-dependent biotin labeling (BioID) coupled with mass spectrometry in M07e cell line.

Results: Transcriptomic analyses in *CG2*-driven mouse AMKL cells identified genes pertaining to pyruvate metabolism, such as pyruvate dehydrogenase kinase 2 (*PDK2*) and pyruvate carboxylase (*PC*). Further investigations showed that 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 in proliferation 7 days post-infection. In addition, treatment with AZD7545, an inhibitor of PDK activity, has a strong effect on proliferation in *CG2*-positive AMKL but also in patient-derived xenograft *in vitro*. In parallel, we identified several partners of transcriptional regulations, suggesting that *CG2* metabolic regulations might occur via gene transcription. By downregulating *CG2* interactors detected in BioID, we observed a decrease in proliferation but also in mitochondrial respiration and glycolysis.

Conclusion: In conclusion, we propose that *CG2* induces metabolic rewiring of hematopoietic cells to promote leukemogenesis. Altered metabolism of cancer cells could create metabolic vulnerabilities that can be targeted with pharmacological inhibitors of metabolic enzymes. None of these vulnerabilities have been previously described in AMKL cell lines and represent novel potential therapeutic targets. Using murine models, in combination with patient-derived cell lines, we seek to identify and validate therapeutic metabolic targets for *CG2*-positive pediatric AMKL. All these experiments will directly support the advancement of optimized clinical protocol that have proven unsuccessful in children with AMKL.

Title: Engineering of natural killer cell against therapy-induced senescent cells

Authors: Joshua Dulong[1,2], Louise Rethacker[2,3], Marie-Ève Lalonde[4], Basma Benabdallah[2], Romain Gioia[2], Kathie Béland[2], Richard Marcotte[3,4], Elie Haddad[2,3,5] et Christian Beauséjour[1,2]

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Keywords: Senescence, Immunotherapy, NK cells, CRISPR screen, iPSC

Background: Cancer treatments (chemotherapy and radiotherapy) or leukemic cells themselves were shown to induce senescence in the bone marrow microenvironment. These senescent cells are non-proliferative but remain metabolically active and can interact with their environment through their secretion phenotype. Although senescent cells can be eliminated by the immune system, including natural killer (NK) cells, it has become evident that a subpopulation employs diverse mechanisms to evade clearance. The accumulation of senescent cells can be deleterious as they enhance cancer cell proliferation and recruitment of immunosuppressive cells (i.e. monocytes). Previous studies showed that senescent fibroblasts can escape NK cell clearance by inducing the expression of the ligand HLA-E and the ganglioside GD3.

Purpose of the study: NK cells are of high interest in immunotherapy since they typically do not induce graft-versus-host disease, making

them the ideal off-the-shelf therapy to target senescent cells. Thus, the aim of this study is to identify immune evasion mechanisms of senescent cells in order to engineer more efficient NK cells.

Methods: To overcome the resistance induced by the binding of HLA-E to its receptor (NKG2A) and the binding of sialic acids present on GD3 to the Siglec-7 receptor, we generated (1) NKG2A knockout (KO) iPSC and NK cells using CRISPR technology, and (2) treated senescent cells with sialidase or used neutralizing antibody targeting Siglec-7.

Results: We first measured the ability of our NKG2A KO NK cells to lyse fibroblasts overexpressing artificially HLA-E. While our engineered NK cells were capable of efficiently lysing these fibroblasts, they were not more competent at lysing ionizing radiation-induced senescent fibroblasts. Likewise, sialidase treatment and neutralizing antibody targeting Siglec-7 could not increase the ability of NK cells at lysing senescent fibroblasts, suggesting that other resistance mechanisms are involved.

Conclusion: We are currently planning a whole-genome CRISPR screen in senescent fibroblasts and in NK cells to identify new mechanisms of immune evasion. Because it is possible to generate multiple genetic alterations at the iPSC level, our study should lead to the development of an ultimate off-the-self senolytic iPSC-derived NK cell therapy.

Title: Subtype-specific Induction of Mitochondrial Apoptosis as a Novel Therapeutic Option 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, apoptosis, BCL-XL, megakaryopoietic lineage

Background information: A rare high-risk subtype of pediatric acute myeloid leukemia (AML) is acute megakaryoblastic leukemia (AMKL). AMKL is considered an infant leukemia (below 3 years of age) with dismal cure rates of <40% and poor prognosis due to resistance to chemotherapy and early relapse. This disease is genetically heterogeneous with recurrent and mutually exclusive oncogenic fusions, such as CBFA2T3::GLIS2 (CG2) or NUP98 rearrangements (NUP98r). The development of targeted therapies is urgently needed but greatly limited by the paucity of primary sample material. Therefore, our research group has established synthetic human models of relevant high-fatality

pediatric AMKL driven by distinct oncogenic fusions. These models reflect the human disease in a patho-physiological relevant context in mice.

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

Methods: Synthetic models of CG2 were generated by transduction of CD34+ cord blood-derived hematopoietic stem and progenitor cells (HSPC) with lentiviral particles carrying the oncogenic fusion and xenografted into immunocompromised NSG mice. This approach generated leukemia that recapitulate human disease regarding immunophenotype, co-mutational landscape, commitment to the mega-erythroid lineage as seen by scRNAseq and gene expression signature, with distinct aberrant expression of pro-survival factor BCL2. Therefore, we investigated the potential of Venetoclax (inhibitor of BCL2) and Navitoclax (inhibitor of BCL2, BCL-XL and BCL-W) as novel therapeutic options due to the aberrant expression of BCL2 in CG2 and commitment of those cells to the megakaryopoeitic lineage, since BCL-XL is an important pro-survival factor of this lineage.

Results: Our results demonstrate that our models of CG2 resist pharmacological and genetic inhibition of pro-survival factor BCL2, which is currently the most targeted BCL-family member in the clinic for AML treatment. In contrast, inhibition of BCL-XL leads to significant induction of apoptosis in our models of AMKL. This was demonstrated by knockdown of BCL-XL as well as treatment with Navitoclax (inhibitor of BCL2, BCL-XL and BCL-W) and a proteasomal degrader of BCL-XL (PROTAC DT2216), with minimal cross-toxicity on normal CD34+ cord blood cells. Additional models of AMKL carrying NUP98r fusions were also vulnerable to inhibition of BCL-XL. Furthermore, treatment with Navitoclax significantly reduced leukemic burden *in vivo* in CG2-xenografted mice in bone marrow and spleen after drug treatment in comparison to vehicle-only controls.

Conclusion: In this study we highlight an exploitable vulnerability of pediatric AMKL cells towards inhibition of pro-survival BCL-XL across genotypes, pointing to a lineage-specific dependency. Furthermore, this project demonstrates the importance of utilising 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.

Title: The receptor Tyrosine Kinase inactivates p53 protein and leads to chemotherapy resistance in leukemia

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Keywords: RTK, MDM2, MDMX, p53, chemoresistance

Background information: Aberrant Receptor Tyrosine Kinase (RTK) signaling allows cancer cells to modulate survival, proliferation and death, leading to tumorigenesis and chemoresistance. In leukemia, one of the frequently deregulated receptors is FLT4 (VEGFR-3), which correlates with the progression of leukemia. However, little is understood about its overexpression and association with drug resistance. Moreover, chemotherapies require the activation of wild type p53 (wt) in tumor cells to induce their apoptosis. Although p53 is rarely mutated in leukemia (5%), it is not understood why its wild type state is non-functional. Indeed, p53 is negatively regulated by the MDM2/MDMX complex, which is overexpressed in leukemia, potentially leading to p53 inactivation. The extracellular signals modulating the MDM2/MDMX complex levels and, therefore, p53 could come from deregulated RTKs to increase the survival of tumor cells.

Purpose of the study: characterize the mechanisms of p53 inactivation by FLT4 in leukemic cells to re-activate p53 function and sensitize leukemic cells to chemotherapy-induced apoptosis.

Methods: First, using HEK293T and U2OS cellular models, FLT4, MDM2 and MDMX vectors were co-transfected to assess the stability and localization of MDM2/MDMX. The MDM2/MDMX complex was immunoprecipitated and analyzed by Mass Spectrometry to determine the protein network modification under FLT4 activation. Second, the ALL leukemic cell line (REH) was 1) treated with the FLT4 specific ligand (VEGF-C) or 2) transduced with a lentiviral vector encoding for FLT4 to induce its constitutive activation and was studied for MDM2/ MDMX stability, p53 expression, survival under chemotherapy treatment (Doxorubicin) and proliferation.

Results: The activation of FLT4 stabilizes the complex MDM2/MDMX. Immunoprecipitation of the heterodimer complex revealed a consensus sequence of CDK4/6 that was phosphorylated when FLT4 was overexpressed, which we confirmed was mediated by CDK4/6 and necessary for MDM2/MDMX stability. The increase of the MDM2/ MDMX complex levels leads to the ubiquitin-induced degradation of p53 and reduction of the expression of its target genes implicated in apoptosis. In addition, FLT4 promotes the relocalization of the complex MDM2/MDMX into

the cytoplasm, limiting p53 transcriptional activity in the nucleus. The activation of FLT4 in the leukemic cells (ligand-dependant or constitutive activation) leads to p53 suppression associated with resistance to chemotherapy-induced apoptosis and increased proliferation.

Conclusion: Acute lymphoid leukemia is characterized by the FLT4/VEGF-C signaling pathway of MDMX/MDM2/p53 axis deregulation. The aberrant activation of FLT4 increases the proliferation and survival of leukemic cells by stabilizing the complex MDM2/MDMX and suppressing p53-mediated cell death .

Title: Taok3 as a novel regulator of B cell activation

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Key words: B cells, adaptive immunity, autoimmunity, B cell lymphoma

Background information: B cell lymphomas are the most common type of blood cancer in adults. They represent a heterogeneous group of blood cancers derived from B cells. Despite medical advances, the specific molecular mechanisms driving certain B cell lymphomas remains unknown, hindering the development of effective treatments. B cells become activated upon antigen recognition through the B cell receptor. Dysregulation of B cell receptor signaling can lead to sustained B cell activation and proliferation. Consequently, several B-cell-derived cancers exhibit dysregulation of several components of the BCR signaling pathway. Moreover, patients with certain autoimmune diseases are at a higher risk of developing B cell lymphomas, highlighting the importance of B cell regulation. Taok3 is a serine threonine protein kinase that is highly expressed in immune cells, and it has been found to be important for T cell receptor signaling regulation. Interestingly, a small study has detected a truncating mutation of Taok3 in a splenic marginal zone B cell lymphoma sample, but the clinical significance of this mutation remains unknown. However, even though one of the highest expressions of Taok3 is found in B cells, little is known about its role in B-cell-related diseases.

Purpose of the study: In our laboratory, we have observed that Taok3 full-body knockout mice exhibit significant dysregulations in B cells. Here, we aim to study the involvement of Taok3 in B cell malignancies, including B cell lymphomagenesis and autoimmunity, focusing on the role that Taok3 may play in B cell activation and BCR signaling.

Methods: Immunophenotyping of Taok3 full-body knockout mice has been carried out using flow-cytometry-based assays, including bone marrow, blood, spleen, lymph node and peritoneal cavity analyses. B cells were isolated from the spleen and lymph nodes of the Taok3 knockout mice and wild-type controls. These cells were stimulated *in vitro* using an anti-IgM monoclonal antibody. Proliferation was analyzed using flow cytometry and the B cell receptor signaling cascade was analyzed using western blotting. Serum levels of immunoglobulins were measured using ELISA, and serum levels of cytokines were measured using multiplex assay.

Results: Taok3 knockout mice display a significant reduction in circulating mature B cells in the bone marrow and blood. *Taok3*$^{-/-}$ mice also present enlarged lymph nodes with increased percentages and numbers of mature B cells, and an increase in the germinal center B cell population, which represents and activated population of B cells. Consequently, memory B cell percentages and numbers were also increased in these mice. Additionally, we observed that Taok3 knockout mice have increased serum levels of immunoglobulins in the absence of infection, specifically the IgM, IgG2b and IgG2c isotypes. Moreover, B cells derived from the lymph nodes of the Taok3 knockout mice showed reduced proliferation *in vitro* at low anti-IgM concentrations compared to wild-type controls, but their proliferation remained unaffected at higher anti-IgM concentrations. Finally, we also observed that *Taok3*$^{-/-}$ mice had increased serum levels of several pro-inflammatory cytokines such as IL-6 and CXCL9.

Conclusions: Taok3 knockout mice display a phenotype consistent with enhanced and sustained B cell receptor signaling. Moreover, we also observed early signs of B-cell-driven autoimmunity, such as the spontaneous formation of germinal centers and increased antibody production in this mouse model. We propose that Taok3 negatively regulates B cell activation by playing a negative role in BCR signaling. Consistent with this, we also bring forward a role for Taok3 in the development of spontaneous autoimmunity, and related B cell lymphomagenesis due to sustained BCR signaling. Understanding how Taok3 contributes to the early onset of autoimmunity and B-cell-derived cancers could be a critical development in the search for a treatment and risk factors for these diseases.

Title: 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: This project aimed 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 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, were co-cultured with dendritic cells (DCs) deriving from mouse bone marrow. After immunomagnetic isolation of the DCs, their repertoire of MHC-I associated peptides (MAPs), called the immunopeptidome, was analyzed by mass spectrometry, with a focus on SILAC labeled peptides.

Results: In spite of intensive work to optimize the conditions of the co-culture, very few cross-presented EL4 peptides have been identified in the immunopeptodime of DCs. They all arise from the translation of canonical regions of the EL4 genome and are not tumor specific. The sensitivity of the current techniques available to analyze the immunopeptidome may not be sufficient enough to study cross-presentation.

Conclusion: Despite the interest of this project to better understand the function of the DCs in tumor immunology and to design more efficient and specific DC-based vaccines targeting TSAs, we face technical limitations and had to stop this project for the moment.

Title: Characterizing central nervous system-infiltrating leukemic cells in pediatric patients

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Keywords: single-cell RNA sequencing, Central Nervous System, Pediatric Leukemia, Cerebrospinal Fluid, Bone Marrow, Acute Myeloid Leukemia

Background information: Central Nervous System (CNS) infiltration by leukemia cells occurs in over 25% of pediatric acute myeloid leukemia (AML) patients. CNS infiltration can manifest with neurological symptoms or can be asymptomatic. It is sought out in all newly diagnosed patients and at relapse by extracting cerebrospinal fluid (CSF) using lumbar puncture and evaluating it under microscope. CNS infiltration confers a higher risk of CNS relapse and is treated with intensified CNS-directed therapy that include intrathecal and systemic chemotherapy. Our knowledge on mechanisms enabling leukemic cells' infiltration of CNS is limited due to their scarcity.

Purpose of the study: We hypothesize that CNS-infiltrating cells have unique biological properties enabling them to infiltrate and survive in the CSF environment, and that these characteristics can be uncovered using single-cell transcriptomics.

Methods: Since 2022, we have been collecting paired bone marrow (BM) and CSF cells from pediatric leukemia patients at CHUSJ at diagnosis or relapse, and processing them using single-cell RNA-sequencing (scRNAseq). We use conventional bioinformatic approaches and our own developed cell type classifier, to investigate the relative abundance of cellular populations and characterize aberrant gene expression and regulatory networks of each of the cellular population between CSF and BM cells.

Results: To this date, we have collected and analyzed 11 CSF and 3 BM samples, and paired our three BM samples with corresponding CSF samples that present leukemic cells infiltrations (AML, n=2, and acute lymphoblastic leukemia, n=1), totaling ~46K cells. We successfully captured leukemic cells in the CSF of all patients that were diagnosed with CNS infiltration in the clinic, enabling us to phenotypically

characterize them. From the 3 paired samples, we observed different abundance in cellular populations between CSF and BM cells in (⅔ patients) and we identified CSF-specific gene expression profiles in paired samples.

Conclusion: scRNAseq is a powerful technique for characterizing CNS-infiltrating leukemic cells at the transcriptomic resolution. Expansion of our patient cohort will allow us to distinguish patient-specific features to those that universally characterize CNS infiltration.

Title: 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 lympho-poiesis as well as in MYC-driven lymphomagenesis, which are important when considering inhibition of DDX3 as a treatment of B-cell lymphoma.

Title: Development of CAR-NK Immunotherapy Using Gene-Edited Induced Human Pluripotent Stem Cells

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

Background information: Autologous CAR-T cell therapy has achieved unprecedented success in treating blood cancer. However, there is a need for an "off-the-shelf" cell product that can be manufactured on a large scale and administered on demand. Natural Killer (NK) cells are innate lymphocytes with potent anti-tumor activity. Since they have MHC-independent cytotoxicity, allogeneic transplantation of NK cells is safe. However, immunosuppressive mechanisms in the tumor microenvironment can hinder NK cell anti-tumor function. Although genome editing of NK cells is a powerful tool for overcoming immunosuppression, technical challenges associated with NK cell gene editing have hampered the use of this approach in the development of immunotherapies. In this regard, induced pluripotent stem cells (iPSCs) can serve as a standardized and inexhaustible source of CAR-NK therapeutics cells, as they can be readily genetically modified and differentiated *in vitro* into NK cells.

Purpose of the study: To develop CAR-NK lymphocytes derived from iPSCs (CAR-iNK) with enhanced anti-tumor activity, we aim to generate iPSC deficient for negative regulators of NK cell cytotoxicity, concomitant with the expression of a CAR before their *in vitro* differentiation into NK cells.

Methods: We will target negative regulators of NK cell cytotoxicity by CRISPR to generate iPSC^{KO} and we will use lentiviral transduction to induce anti-CD22 CAR transgene expression in modified iPSCs (CAR-iPSC^{KO}). Subsequently, CAR-iNK cells will be generated through cytokines directed *in vitro* differentiation and expanded using feeder cells. Their anti-tumor activity will be assessed *in vitro* against different leukemia cell lines; K562, NALM6, 697, and REH. Finally, we will establish proof of concept for our approach by testing the antitumor activity of CAR-iNK cells *in vivo* in a xenogeneic mouse model.

Results: We evaluated the feasibility of our approach in primary NK cells by using CRISPR to target *KLRC1*, which encodes NKG2A, a potent negative regulator of NK cells anti-tumor activity. Our findings confirm the relevance of targeting NKG2A, as *KLRC1*^{KO} NK cells demonstrate enhanced anti-tumor activity against four types of cancers *in vitro*. When adoptively transferred to a xenogeneic mouse model of metastatic cancer, *KLRC1*^{KO} NK cells significantly delayed tumor progression and prolonged survival compared to wild-type NK cells (p=0.0015). We generated both *KLRC1*^{KO} and anti-CD22 CAR iPSCs. Moreover, we were able to generate mature NK cells from iPSCs, and our results show that both genetically edited iPSCs retain their ability to differentiate into NK cells. Preliminary results suggest a synergistic effect between the expression of CAR and the deletion of *KLRC1* in enhancing NK cell anti-tumor activity and we are currently investigating various CARs in combination with our strategy.

Conclusion: There is an urgent need to improve accessibility to cutting-edge treatment, such as cellular immunotherapy. CAR-iNK holds the potential to shift this complex personalized therapeutic option to a universal "off-the-shelf" one. The versatility offered by iPSCs allows for the application of this strategy to other malignancies. Moreover, by studying the loss of function of the targeted negative regulators, we can gain valuable insights and deepen our understanding of the mechanisms underlying NK cell-mediated anti-tumor responses.

Title: Genome-wide CRISPR screen identifies factors influencing Natural Killer (NK) cell activity against NK-resistant tumor models

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

Background information: B-cell acute lymphoblastic leukemia (B-ALL) is the most common type of cancer in children, and while chemotherapy can be successful, long-term side effects remain a concern. Ongoing research is exploring targeted and less toxic therapies for B-ALL in children. Natural killer (NK) cells are innate lymphocytes that demonstrate cytotoxic activity towards stressed-self and/or modified-self cells, such as cancer cells. NK cells demonstrate strong anti-leukemic activity, and the presence of NK cells infiltrating the bone marrow is linked to favorable outcomes in B-ALL patients. Nevertheless, some patients

experience reduced NK cell function due to resistance mechanisms and NK cell exhaustion.

Purpose of the study: To develop new therapies or improve existing ones, we need to fully understand how NK cells respond and kill B-ALL cells and what are the genes and pathways involved in this response.

Methods: In order to identify key genes regulating sensitivity or resistance to NK cell cytotoxicity, we performed a whole-genome CRISPR screen in 697 B-ALL cell line. Using the Yusa CRISPR library, we induced mutations resulting in the knock out (KO) of 18,010 genes in 697 cells. Then, we co-cultured those cells with primary activated NK cells, derived from the NK-cell Activation and Expansion System (NKAES), until we reached more than 80% of target cell death. In previous cytotoxic assays, we showed that 697 cells are resistant to NK cell lysis after 4h of co-culture but become more sensitive after 24h of co-culture. Considering these results and to reach 80% of cytotoxicity, we did two rounds of 24h of co-culture at a ratio 4:1.

Results: First, the MAGeCK Robust Rank Analysis in surviving cells identified the death receptor pathway as the major mechanism involved in the NK cell mediated lysis of 697 cells. In fact, sgRNA targeting *FADD*, *CASP8*, *BID*, *FAS* and *TNFRSF10A* were enriched in resistant tumor cells. We generated the 697 cell line KO for *FADD* and confirmed with a cytotoxicity assay that loss of FADD decreased NK cell-mediated killing. Furthermore, as expected the analysis of the depleted sgRNA in surviving 697 cells showed that loss of *HLA-E, HLA-C* and *B2M* increase their sensitivity to NK cells. HLA molecules are known ligands of NK cell inhibitory receptors, HLA-E is a ligand of NKG2A and HLA-C is recognized by the killer cell immunoglobulin receptors (KIRs).

Conclusion: In summary, the preliminary results of our study indicate that inducing the death receptor pathway or NKG2A and/or KIR receptors blockade could potentially improve the effectiveness of NK cell-based immunotherapy in resistant B-ALL. We are presently applying the same approach to investigate additional killing pathways and resistance mechanisms in other NK-resistant B-ALL cell lines. Through this screening method, we aim to develop novel NK-based immunotherapies for B-ALL and various other cancer types.

Title: Enhancement of the graft-versus leukemia effect through the modulation of Neuropilin-1 expression in the context of allogeneic hematopoietic stem cell transplant

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Keywords: Leukemia – Graft – Immunotherapy – Neuropilin-1

Background information: Neuropilin-1 (NRP1) is a membrane co-receptor highly prevalent in tumors that is associated with the immunosuppression mechanisms leading to tumor escape. NRP-1 is expressed in a multitude of immune cell populations (myeloid and lymphoid). It has been demonstrated that NRP-1 is expressed on populations with immunoinhibitory properties such as regulatory T lymphocytes (Treg) and macrophage subtypes and that its deletion attenuates the suppressive effects. We also find NRP-1 on activated effector CD8 T cells and its expression can lead to a defect in their effector functions. NRP-1 does not render CD8 completely dysfunctional, but rather appears to promote an exhaustion phenotype. The exact mechanisms of modulation of CD8 activity by NRP-1, however, remain to be elucidated. An increase in the expression of NRP-1 has also been observed in leukemic cells and in the bone marrow of patients with AML and this expression has been associated with a poor prognosis. The most common treatment for AML is chemotherapy, which may be accompanied by a hematopoietic stem cell transplant (SCT). The most common SCT is the allogeneic transplant which consists of using stem cells from a partially histo-incompatible donor. Allogeneic transplantation comes with high risks of GVHD but also the potential to induce GVL and protect the patient from relapse.

Purpose of the study: By first elucidating the function of NRP1 in various immune cells and tumor models, our project aims to modulate NRP1 expression in the context allo-HSCT in a way that will enhance the GVL effect while limiting the risk and severity of GVHD.

Methods: We assess the expression of NRP1 at different stages of T cell activation in vitro and assess the impact of NRP-1 overexpression in CD8 using a retroviral vector to better observe its inhibitory effects. We use flow cytometry to characterize T cells and mice models to define the role of NRP-1 in tumor and transplant immunology.

Results: Our preliminary data shows that NRP1 is induced only upon activation of CD8 T cells and its expression is directly proportional to the intensity of the activating signal. NRP1 does not seem to have any effect on the effector functions of CD8 T cells even when incubated with known NRP1 ligands. The expression of NRP1 correlates with the

expression of the exhaustion markers PD-1 and 2B4. NRP1 is induced in the tumor microenvironment and is highly expressed in tumor infiltrating CD8 T cells. Our retroviral vector has shown great transduction efficiency as evidenced by a 3-fold increase in surface expression of NRP1. When adoptively transferred into tumor-bearing mice, T cells transduced with the NRP1 vector show a better infiltration compared to an empty vector control.

Conclusion: NRP1 expression is highly correlated with activation, intra-tumoral infiltration and expression of exhaustion markers in CD8 T cells. We anticipate that NRP-1 expression will also correlate with tissue and tumor infiltration in the context of transplantation to treat leukemia/lymphoma. Future studies will determine whether NRP-1 gain or loss of function impacts GVHD and GVL.

Title: Biophysical characterization of oncogenic RAS signaling in living cells using in-cell NMR

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Keywords: RAS GTPases, oncogenic mutants, NMR spectroscopy, structural biology

Background information: RAS small GTPases alternate between GDP-bound inactive and GTP-bound active states, which permits effector binding and thereby orchestrate multiple cellular responses including proliferation and may induce cell death. RAS mutations are found in almost 30% of all human cancers, and two out of three RAS isoforms, NRAS and KRAS, are frequently mutated in Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML) and Juvenile Myelomonocytic Leukemia (JMML).

Purpose of the study: To date, all biophysical data of RAS activity comes from in vitro assays. This is a major limitation considering the complexity of RAS-effector signaling pathways in living cells. Thus, we require innovative approaches to study these proteins in their native environment.

Methods: Real-time monitoring of protein conformation using NMR provides detailed, atomic-level insights of enzymatic activity and protein-protein interactions. In-cell NMR spectroscopy has the potential to move these analyses into living cells, whereby we monitor the activation and biochemical modifications of RAS and its downstream partners in real time. This is achieved by transducing uniformly (^{15}N) or specifically (^{13}C-Isoleucine) isotopically labelled protein purified from *E. coli* into living mammalian cells through electroporation.

Results: Our current results show that GTPases are stable in cells after transduction. We can visualize transduced proteins with microscopy and/or by Western Blots in HEK-293T, HeLa, Nalm-6 and THP-1 cell lines. NMR spectroscopy allows the detection wild-type and mutant variants of GTPases in living HEK-293T cells. Notably, we can differentiate the active and inactive GTPase states using in-cell NMR.

Conclusion: In-cell NMR will allow us to elucidate how RAS GTPases cycle between active and inactive states, how oncogenic mutations perturb cycling and the mechanisms that dictate signaling output in cells. Detailed biophysical characterization of these key proteins in living cells will help us to develop novel anti-RAS therapies and ultimately improve outcomes for cancer patients.

Title: PVRIG: A Novel Immune Checkpoint with Immunological and Prognostic Implications in Pan-cancer Uncovered through Integrated Bulk and Single-Cell Sequencing Analyses with Experimental Validation

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Keywords: PVRIG, prognosis, immune checkpoint, pan-cancer, immunotherapy

Background information: Poliovirus receptor-related immunoglobulin domain-containing (PVRIG) is a newly identified immune checkpoint receptor mainly expressed in natural killer (NK) and T cells. PVRIG blockade and deficiency in mice and humans can enhance the antitumor activity of NK and T cells. However, as a newly reported immune checkpoint receptor, the role of PVRIG within the tumor immune microenvironment and its relation to prognosis across multiple cancer types is still not fully understood.

Purpose of the study: This study aimed to explore the biological functions of PVRIG and its impact on tumor heterogeneity, immune response, and prognosis.

Methods: A pan-cancer analysis was performed to evaluate the impact of PVRIG expression on the patient prognosis, immune infiltration, and expression of immune modulator and immune checkpoint genes. We also assessed the expression of PVRIG on tumor-infiltrating lymphocytes (TILs) in multiple cancers through single-cell sequencing analysis. The expression of PVRIG on NK and CD8+ T cells and its co-expression with exhausted markers PD-1 and TIM3 in mouse melanoma models were explored using flow cytometric analysis. Moreover, the associations between PVRIG expression and tumor heterogeneity, stemness,

and ultimately the response to immunotherapy and small molecule targeted drugs were investigated. Gene set enrichment analysis (GSEA) was used to assess the biological functions of PVRIG.

Results: PVRIG was differentially expressed in most tumor types and differently associated with clinical prognosis among different tumor types. PVRIG expression was closely associated with many tumor-infiltrating immune cells, such as CD8+ T cells, NK cells, M1 and M2 tumor-associated macrophages (i.e., M1- and M2-TAMs), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs). Moreover, PVRIG expression was positively correlated with the immune, ESTIMATE, and stromal scores in multiple types of human tumors, and also positively correlated with the expression of the most immune modulator and immune checkpoint genes. Single-cell sequencing analysis revealed that PVRIG was highly expressed on exhausted CD8+ T cells. Furthermore, flow cytometric analysis revealed that PVRIG was positively correlated with exhausted markers PD-1 and TIM3 in NK and CD8+ T cells in mouse melanoma tumors. GSEA analysis showed that PVRIG was tightly associated with T cell functions, including T cell activation and differentiation. Finally, PVRIG demonstrated a good predictive ability for response to immune checkpoint blockade (ICB) therapies and multiple small-molecule targeted drugs.

Conclusion: PVRIG could be used as a novel biomarker to predict immune infiltration and prognosis in multiple tumors. It could also potentially be used as a new immune checkpoint target for novel tumor immunotherapies.