Jeanette Boudreau, PhD

Jonathan Bramson, PhD

Marcus Butler, MD

Tracy McGaha, PhD

John Ronald, PhD

Ian Watson, PhD

4TH ANNUAL

IMMUNO-ONCOLOGY SYMPOSIUM

CANCER IMMUNOLOGY, CANCER IMMUNO-IMAGING, AND CANCER IMMUNOTHERAPY

WEDNESDAY, MARCH 29, 2023

8:30AM TO 5:30PM

GREAT HALL, SOMERVILLE HOUSE

4th Annual Immuno-Oncology Symposium
Wed, March 29, 2023
8:30 to 17:30 EST

Symposium Booklet

@WesternU_CTCR #CTCRIO2023	
8:30 – 9:00	**Arrival & Networking**
9:00 – 9:10	**Welcome and Opening Remarks** **Saman Maleki, PhD**. Conference Chair **John Ronald, PhD.** Conference Co-Chair
9:10 – 10:50	***SESSION #1***
9:10 – 9:50	***"Approaches to Engineering Anti-Tumor Immunity."*** **Marcus Butler, MD** Medical Oncologist, Princess Margaret Cancer Centre. Clinical Investigator, University Health Network Research. Assistant Professor, Department of Medicine, and Associate Member, Department of Immunology, University of Toronto.
9:50 - 10:30	***"Triggering the magic bullet: How synthetic antigen receptors influence T cell therapy."*** **Jonathan Bramson, PhD** Vice Dean, Health Sciences, Research, Faculty of Health Sciences. Professor, Department of Pathology and Molecular Medicine, McMaster University. Tier 1 Canada Research Chair in Translational Cancer Immunology and the John Bienenstock Chair in Molecular Medicine.
10:30 – 10:50	***Trainee Talks*** ***1."The role of NKR-P1B receptor in regulating NK cell responses in breast cancer."*** Karla Alnajm, MSc. Candidate ***2. "Novel population of CD103+ regulatory innate lymphoid cells suppress intratumoural T cells in patients with epithelial ovarian carcinoma."*** Douglas Chung, PhD Candidate
10:50 – 11:10	**Health Break**
11:10 – 12:50	***SESSION #2***
11:10 – 11:50	***"Identification of strategies to overcome immunotherapy resistance in melanoma using multi-omic approaches."*** **Ian Watson, PhD** Member, Rosalind and Morris Goodman Cancer Institute. Investigator, Research Institute-McGill University Health Center (RI-MUHC). Canada Research Chair II in Functional Genomics of Melanoma. Associate Professor, Department of Biochemistry, McGill University

11:50 – 12:30	***"Imaging Cellular Immunotherapies with Reporter Gene-Based Technologies".*** **John Ronald, PhD** Associate Professor, Department of Medical Biophysics, and Oncology, Schulich School of Medicine & Dentistry, Western University. Scientist, Robarts Research Institute. Associate Scientist, Lawson Health Research Institute
12:30 – 12:50	***Trainee Talks*** ***3. "Target-dependent expression of synthetic blood biomarker in detecting in vivo cell-cell communication."*** *Yanghao (Jerry) Fu,* MSc. Candidate ***4. "Anti-CTLA-4 efficacy against neuroblastoma tumours with induced DNA mismatch repair deficiency relies on Fc-dependent depletion of Tregs."*** *Megan Hong,* MSc. Candidate
12:50 – 14:15	**Lunch Break & Poster Session**
14:15 – 15:55	***SESSION #3***
14:15 – 14:55	***"Natural killer cells: consistent allies and rising stars in cancer therapy."*** **Jeanette Boudreau, PhD** Cameron Cancer Chair, and Scientific Director, Beatrice Hunter Cancer Research Institute. Associate Professor, Division of Medicine, in the Departments of Microbiology & Immunology, and Pathology, Dalhousie University.
14:55 - 15:35	***"Tryptophan metabolism, the microbiome, and innate immunity in the tumor microenvironment."*** **Tracy McGaha, PhD** Senior Scientist, Tumor Immunotherapy Program, Princess Margaret Cancer Centre University Health Network. Professor, Department of Immunology, University of Toronto.
15:35 – 15:55	***Trainee Talks*** **5.** ***"Modeling THP-1 monocyte migration using iFlowPlate™ in an in-vitro cancer model."*** Mandeep Marway, PhD Candidate ***6. "PU.1 Inhibition restrains tumor growth by promoting the CXCL9/10/11-CXCR3 network in the B16-OVA melanoma mouse model."*** Nichita Sleapnicov, *MSc. Candidate*
15:55 – 16:15	**Closing Remarks & Award Presentations**
16:15 – 17:30	***Networking***

THE ROLE OF NKR-P1B RECEPTOR IN REGULATING NK CELL RESPONSES IN BREAST CANCER

Karla Alnajm[1], Mohamad B. Alkassab[1], Mary Ibrahim[1], Mir Munir A. Rahim[1]
[1]Department of Biomedical Sciences, University of Windsor, Windsor, Ontario

Natural killer (NK) cells are innate lymphocytes that respond to diseased cells via two effector functions: direct cytotoxicity; and release of immunomodulatory cytokines. Target cell recognition is mediated by an overall signaling balance of activating and inhibitory receptors, which recognize ligands on target cells. The inhibitory NKR-P1B receptor, expressed on mouse NK cells, recognizes the C-type lectin-related protein-b (Clr-b) ligand and mediates a 'missing-self' response against cells lacking Clr-b. We have shown an important role for NKR-P1B in cancer immunosurveillance in MMTV-PyVT mouse model of breast cancer. Here, we describe the role of NKR-P1B:Clr-b interaction in immunoevasion and NK cell homeostasis in breast cancer. Injection of E0771 mammary adenocarcinoma cells into the mammary fat pad of female C57BL/6 mice gives rise to Clr-b$^+$ mammary tumours. Clr-b is also expressed in tumour-infiltrating leukocytes (TIL). Using E0771 and Clr-b-deficient E0771, generated by CRSPR-Cas9 mutagenesis, we have induced mammary tumours in WT, *Nkrp1b*$^{-/-}$, and *Clr-b*$^{-/-}$ mice to study cancer immunoevasion via NKR-P1B:Clr-b interactions. E0771 tumours grew slower and fewer *Nkrp1b*$^{-/-}$ mice developed mammary tumours compared to WT mice. Tumour growth was intermediate in *Clr-b*$^{-/-}$ mice, which lack Clr-b expression in TILs only. Flow cytometric analysis revealed higher frequencies of PD-1$^+$ effector EOMES$^+$CD49a$^+$ NK cells in mammary tumours from *Nkrp1b*$^{-/-}$ compared to WT and *Clr-b*$^{-/-}$mice, possibly due to their higher activity. In i*n vitro* co-culture assays, E0771 mammary tumour cells induce activation and proliferation in both WT and *Nkrp1b*$^{-/-}$ NK cells. However, larger proportions of *Nkrp1b*$^{-/-}$ NK cells attained an effector EOMES$^+$CD49a$^+$ phenotype than WT NK. These experiments indicate that Clr-b expression in both tumour cells and TILs can contribute to immunoevasion via NKR-P1B. *In vivo* and *in vitro* experiments using Clr-b-deficient E0771 cells will further highlight the contribution of tumour cells and TIL in immunoevasion via NKR-P1B:Clr-b axis in breast cancer.

Novel population of CD103+ regulatory innate lymphoid cells suppress intratumoural T cells in patients with epithelial ovarian carcinoma

Douglas Chung[1,2], Jehan Vakharia[2] , Kathrin Warner[2] , Nicolas Jacquelot[2] , Azin Sayad[2] , SeongJun Han[1,2], Maryam Ghaedi[2] , Carlos Garcia-Batres[2] , Alisha R Elford[2] , Jessica A Matthews[1,3,4], Ben X Wang2 , Linh T Nguyen[2] , Patricia A Shaw[5] , Blaise A Clarke[5,6], Marcus Q Bernardini[5,6], Sarah E Ferguson[5,7], Sarah Q Crome[1,3,4], and Pamela S Ohashi[1,2]

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[2]Princess Margaret Cancer Centre,
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[5]Division of Gynecologic Oncology, University Health Network,
[6]Department of Laboratory Medicine and Pathobiology, University of Toronto,
[7]Department of Obstetrics and Gynecology, University of Toronto

Immunotherapy have had unprecedented success in select tumour types, albeit with limited clinical response rates. Further understanding on immunoregulatory networks may provide insights and novel targets to enhance anti-tumour immune response. Regulatory innate lymphoid cells (ILCregs) are an emerging family of immunoregulatory cells that suppresses innate and adaptive immunity in disease contexts including cancer. Currently, there is a lack of lineage-defining features and tools that distinguishes ILCregs from canonical ILCs with cytolytic functions. In this study, we identified CD103 expressing $CD56^+$ ILCreg that have distinct properties from canonical $CD56^+$ NK cells. Moreover, $CD103^+$ ILCregs were associated with poor clinical prognosis, slow growing patient-derived tumour-infiltrating lymphocyte (TIL) cultures, and poorly activated T cell phenotype *ex vivo*. $CD103^+$ ILCregs expressed surface markers (i.e., CD39, CDCD69, TIGIT, and CD101) and transcription factor regulatory networks (i.e., PRDM1, ETS1) that play roles in Treg stability, development, and suppressive functions. $CD103^+$ ILCregs also suppressed autologous tumour-infiltrating CD8+ T cells *in vitro*. Finally, we found that ascites-derived TGF-β induced NK-to-$CD103^+$ ILCregs conversion. Overall, we identified a novel population of ILCregs in the TME that play a role in suppressing intratumoural T cells. These findings may provide novel tools in understanding ILCreg biology and may be a key target for future combination therapies. Moreover, the role of TGF-β induced NK-to-ILCreg conversion raises interesting implications on our understanding about NK dysfunction and current NK-based cellular therapies.

Target-dependent expression of synthetic blood biomarker in detecting *in vivo* cell-cell communication

YangHao Fu[1,2], TianDuo Wang[1,2], John. A. Ronald[1,2,3]
1. Robarts Research Institute – Imaging; 2. Department of Medical Biophysics, University of Western Ontario; 3. Lawson Health Research Institute, London, ON, Canada.

INTRODUCTION: The field of cellular immunotherapy has greatly improved cancer treatment over recent years. For instance, chimeric antigen receptor (CAR) T-cell therapy has been proven highly effective in treating blood-based cancers such as leukemia and lymphoma in most patients.[1] However, it is still difficult to understand why they have minimal effect in others. Clinically, blood tests are regularly used to monitor immunotherapies through circulating immune cells, which does not capture treatment efficacy. Here, we built a new activatable system that secretes a unique blood biomarker when engineered therapeutic immune cells interact with target cancer cells within tumors. The Synthetic Notch (SynNotch) receptor can signal cell-cell contact via transcriptional modulation of desired genes in response to SynNotch receptor-antigen binding (Fig. 1A).[2] Our approach is to engineer immune cells with a SynNotch receptor that, upon cancer antigen binding, activates the expression of secreted embryonic alkaline phosphatase (SEAP) – a safe and sensitive human-derived blood-based reporter[3], allowing for blood-based detection of *in vivo* cell-cell communication.

METHODS: We engineered a human T cell line, via sequential lentiviral transduction of two components: (1) a SynNotch receptor directed against the B cell leukemia antigen CD19, and (2) a reporter response element encoding SEAP. The B cell surface antigen CD19 was chosen as it is the most successful target of CAR-T immunotherapy in humans currently.[5] Successfully dual-engineered T cells were isolated using fluorescence-activated cell sorting and expanded. To validate this activatable system *in vitro*, 10^5 T cells were co-cultured in well plates with CD19+ B cells at a 1:1 ratio. As a negative control, CRISPR-knockout was used to generate CD19-B cells. In media, SEAP concentration was assessed every 24 hours for 2 days. Translating this system *in vivo*, Nod-scid-gamma (NSG) mice were implanted with either CD19+ or CD19- Nalm6 cells subcutaneously. Once tumours reached ~100m^3, mice received an intratumoural injection of engineered T cells (1x10^7), and blood samples were taken from the left flank for SEAP assays.

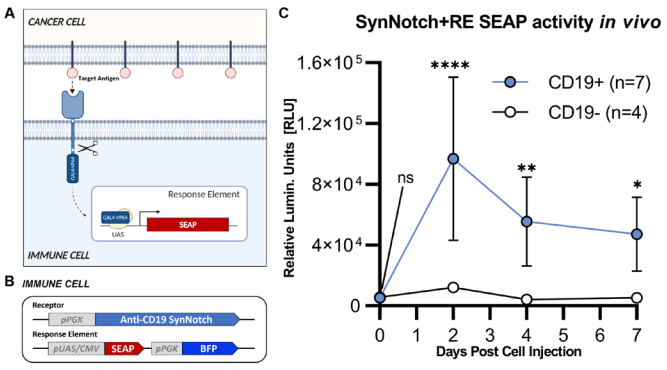

Figure 1. (A) Schematic of SynNotch system. CD19 binding by the CD19-targeted SynNotch receptor induces intracellular cleavage of a GAL4- VP64 transactivator, which binds to an upstream activating sequence (UAS) to initiate transcription of genes of interest encoded in the response element. **(B)** T cells were engineered with CD19- targeted SynNotch driven by the phosphoglycerate kinase 1 promoter (pPGK), and a response element containing reporter genes as well as a pPGK-driven blue fluorescence protein (BFP) for sorting. **(C)**Average blood SEAP activity measured from mousecarrying CD19+ or CD19- tumor (B) cells before and after the delivery of SynNotch+RE T cells.

RESULTS: *In vitro* co-culturing of dual-engineered T cells with CD19+ cells resulted in significantly higher SEAP activity on all days ($p < 0.001$). In contrast, reporter expression was minimal when T cells engineered with only the reporter gene or naïve T cells when co-cultured with CD19+ cells. Co-culturing with CD19- cells resulted in no detectable SEAP levels above the background. *In vivo*, no SEAP activity was observed prior to engineered cell injection in tumor-bearing mice (Fig. 1C). Importantly, significantly elevated SEAP activity was observed post-T cell injection in mice bearing CD19+ tumors, but not CD19- tumors (Fig. 1C).

DISCUSSION: We have established a synthetic biology reporter system that is antigen activatable, which allows for the detection of immune-cancer cell interactions through a simple and convenient blood test. Future work includes transferring this system into primary T cells as well as expanding the potential target antigen. The development of this system would allow for a specific monitoring tool for many cell-based cancer immunotherapies, ultimately improving our understanding of the treatment dynamics as well as the presence of side effects in various individuals.

REFERENCES: [1] June et al., Science, 2018, 359(6382):1361-1365. [2] Maude et al., NEJM, 2014, 371: 1507-1517. [3] Berger et al., Gene, 1988, 66(1):1-10. [4] Sadelain et al., J Clin Invest, 2015, 125(9): 3392-3400.

Title: Anti-CTLA-4 efficacy against neuroblastoma tumours with induced DNA mismatch repair deficiency relies on Fc-dependent depletion of Tregs

Authors: Megan M Y Hong, Rene Figueredo, Saman Maleki Vareki

Introduction: Expression of immune checkpoint molecules such as CTLA-4 on T-cells can suppress anti-tumour immune responses by inhibiting T-cell activation and function. Anti-CTLA-4 is an immune checkpoint inhibitor (ICI) that enhances patients' anti-tumour immune responses to eliminate cancer cells. ICIs have revolutionized the treatment of cancer in the last decade; however, their use is limited by their efficacy to only a small fraction of patients.

The DNA mismatch repair (MMR) pathway corrects mismatched base pairs that occur during DNA replication. Notably, patient response to ICIs is positively associated with those with MMR-deficient (dMMR) tumours in several solid cancers. However, studies have not addressed why dMMR tumours are more sensitive to ICIs than their pMMR counterparts. We have previously shown that inducing MMR deficiency in ICI-refractory and MMR-proficient (pMMR) neuroblastoma tumours rendered them sensitive to anti-CTLA-4 therapy. This study investigates the importance of anti-CTLA-4-mediated regulatory T-cell (Treg) depletion in its efficacy against induced dMMR (idMMR) neuroblastoma tumours.

Methods: MMR repair deficiency was induced in the murine neuro-2a cell line by knocking out *MLH1* expression using CRISPR/Cas9. pMMR or idMMR tumours were grown in immunocompetent syngeneic mice and treated with anti-CTLA-4 once tumours were palpable. Tumour growth was measured followed by immunophenotyping of tumours and spleens by flow cytometry.

Results: Anti-CTLA-4 therapy increased $CD3^{+}$ cells and decreased Tregs in idMMR neuroblastoma tumours but had no effect on pMMR tumours. idMMR tumours had an increase of macrophages compared to pMMR tumours and FcγRIV expression was increased on pro-inflammatory $Ly6c^{High}$ macrophages. Treatment with an anti-CTLA-4 clone that does not have an affinity towards FcγRIV did not deplete Tregs in idMMR tumours and failed to control tumour growth.

Discussion: These results suggest that the therapeutic effect of anti-CTLA-4 against idMMR tumours is mediated by Fc-dependent mechanisms that deplete Tregs. The increase of macrophages and FcγRIV expression may enhance anti-CTLA-4-mediated Treg depletion in idMMR tumours. These results further our understanding of the biology of pMMR and dMMR tumours and tumour immune microenvironment features that facilitate ICI sensitivity. Targeting the MMR pathway may be a therapeutic approach to improve ICI response in patients with ICI-refractory tumours.

Modeling THP-1 monocyte migration using iFlowPlate™ in an in-vitro cancer model

Mandeep Marway

abstract omitted by author

PU.1 INHIBITION RESTRAINS TUMOR GROWTH BY PROMOTING THE CXCL9/10/11-CXCR3 NETWORK IN THE B16-OVA MELANOMA MOUSE MODEL

Nichita Sleapnicov
Department of Microbiology & Immunology, University of Western Ontario, London, ON.

The tumour immune microenvironment (TIME) plays an essential role in the growth and development of tumors. Within the TIME, tumour-associated macrophages (TAMs) have an immune-suppressive phenotype, playing a key role in immune suppression and tumour growth. TAMs are also one of the most abundant immune cell populations in solid tumours. TAMs are therefore an ideal anti-tumour immunotherapy target, but TAM-selective reagents are still to be discovered. The ETS-family transcription factor PU.1 is crucial for the generation of macrophages, but its role in TAM differentiation and activity is poorly understood. To address this question, we examined transcriptomic changes in response to PU.1 inhibitor DB2313 (DB) in LPS- and IL4-activated bone marrow-derived macrophages (BMDMs). We found that DB inhibited the expression of both pro-inflammatory and immunosuppressive cytokines and markers, whereas the expression of several chemokines including CXCL9/10/11 notably increased. These chemokines and their receptor, CXCR3, are an important network for immune cell recruitment. To examine the antitumour effects of DB, C57BL/6 mice bearing B16-OVA melanoma cells were treated with DB and/or a blocking antibody against CXCR3. DB alone significantly restrained the tumor growth, an effect abrogated by CXCR3 antibody. Bulk RNA-sequencing of the tumours, followed by gene ontology enhancement analysis by Cytoscape and in-silico deconvolution via CIBERSORT showed that DB enhanced recruitment of CD4+/CD8+ T cells and antigen-presenting DCs in tumours. Collectively, these results suggest that inhibition of PU.1 enhances anti-tumor immune responses likely through promoting the CXCL9/10/11-CXCR3 network in TIME. This study investigates PU.1 as a potential target for anti-tumor immunotherapy.

Poster Presentation Abstracts

Poster Num.	First Name	Last Name	Training Level
7	Sawyer	Badiuk	UWO PhD Candidate
8	Michael	Celejewski	McMaster Master's Student
9	Lianghong	Chen	UWO Undergraduate Thesis Student
10	Andris	Evans	UWO PhD Candidate
11	Hasti	Gholami	UWO Master's Student
12	Mary	Ibrahim	Windsor Master's Student
13	Jessica	Jeong	UWO Undergraduate Thesis Student
14	Frederikke	Larsen	UWO PhD Candidate
15	Amanda	Liddy	UWO Master's Student
16	Allanna	MacKenzie	UWO Master's Student
17	Vitoria	Olyntho	McMaster Undergraduate Thesis Student
18	Sydney	Relouw	UWO Master's Student
19	Noor	Salloum	UWO PhD Candidate
20	Rafael	Sanchez-Pupo	UWO Post Doc.
21	Nourhan	Shalaby	UWO PhD Candidate
22	Danielle	Taray-Matheson	UWO Master's Student
23	Saurav	Verma	UWO Medical Oncology Fellow
24	Cathy (Wei Cen)	Wang	UWO 2nd Year Medical Student
25	TianDuo/Jerry	Wang/Fu	UWO PhD Candidate
26	Ying	Xia	UWO Research Associate
27	Sissi (Zi)	Yang	McMaster's Master Student
28	Rada	Tazhitdinova	UWO Master's Student

Poster #7

Imaging glial activation using [18F]-FEPPA PET post-cranial irradiation

Badiuk S, Qi Q, McClennan A, Desjardins L, Fox M, Foster P, Thiessen J, Chen J, Wong E.

Department of Medical Biophysics and Physics and Astronomy, University of Western Ontario, Lawson Health Research Institute, Robarts Research Institute, London Regional Cancer Centre.

Introduction

Brain metastases (BM) have been effectively treated with stereotactic radiosurgery (SRS) delivered to visible growths followed by whole brain radiotherapy (WBRT) for microscopic disease. SRS alone is the preferred treatment despite high recurrence rates, as conventional WBRT is associated with increased cognitive decline. With improved systemic treatments, breast cancer patients are living longer which challenges the decision to withhold WBRT. Cognitive decline has been linked to chronic inflammation and radiation induces inflammation via glial cell (microglia, astrocytes) activation. When glial cells are activated, translocator proteins (TSPO) on the mitochondria upregulate and promote inflammation. Glial activation has been assessed in neurological disease using a FEPPA ligand with high affinity for TSPO with positron emission tomography (PET). The aim of this pilot study is to investigate the potential application of glial activation imaging using the novel radiotracer [18F]-FEPPA and PET imaging with brain irradiation in a murine model. This work presents the studies preliminary imaging data.

Methods

To evaluate radiation induced glial activation, brain irradiation was performed on non-tumour bearing immunocompetent mice (BALB/c)(N=16) using a micro-CT/RT system with 4, 8, 10 and 15Gy. [18F]-FEPPA-PET was completed at 24hrs, 48hrs, or 2 weeks after irradiation to quantify level and duration of glial activation. Dynamic [18F]-FEPPA-PET images were acquired for 90 min, with tail vein injection of 10-20MBq of [18F]-FEPPA. PET kinetic analysis was performed on each scan to compute time activity curves and volume of distribution (Vt). Standard uptake value (SUV) was also employed in our analyses. Currently, [18F]-FEPPA-PET has been used to evaluate half-brain irradiation (N=4) with 10Gy and 15Gy and whole brain irradiation (N=12) with 4, 8, 10 and 15Gy. Autoradiography was performed on half of the mice. Immunohistochemistry identifying glial activation was performed with peripheral benzodiazepine (PBR) staining TSPO and ionized calcium binding adaptor molecule 1 (IBA1) staining for activated microglia.

Results

Preliminary assessment of Vt and SUV for half-brain irradiation quantitatively showed similar values in the irradiated side of the brain compared to the contralateral side at all time points, with a representative PET image at 48 hours after irradiation shown in Figure 1.B. Sample autoradiography (Figure 1.C) and TSPO staining (Figure 1.D) at 48 hours after half-brain irradiation also demonstrated similar signal between hemispheres. Vt assessment for WBRT was increased at the 48 hrs time point compared to 24 hrs and 2 weeks for all dose levels. Higher Vt was evident for low dose levels compared to the high dose levels at earlier imaging timepoints (24hrs, 48hrs) for WBRT but return to similar values at 2 weeks.

Discussion

This work demonstrated that partial brain irradiation induces global glial activation and that dose and duration post-irradiation effect glial activation. Further investigation is required before making any conclusions,

however this pilot study will validate [18F]-FEPPA as a suitable radiotracer for evaluating glial activation post-irradiation. Following this pilot study, [18F]-FEPPA-PET and MRI single cell tracking will be used in a breast cancer BM model to discover an alternative WBRT treatment that optimizes breast cancer BM control with reduced cognitive decline.

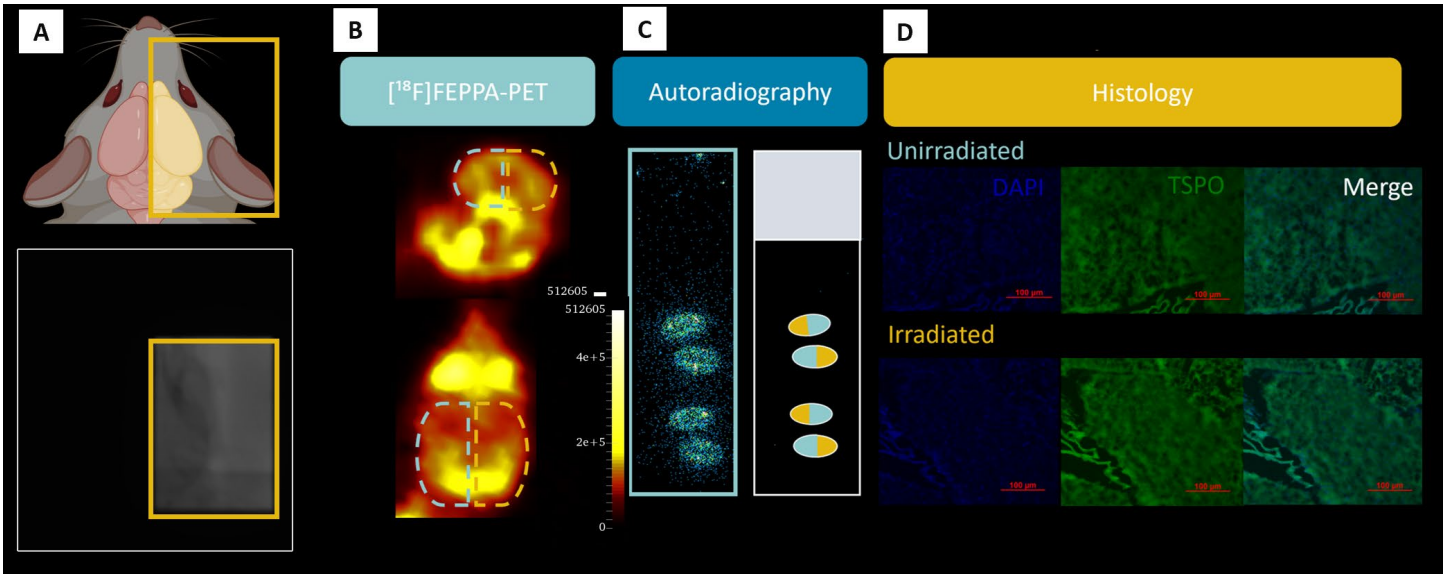

Figure 1. Diagrams and images for half-brain irradiation of BALB/c mouse that received 15 Gy imaged 48 hours later. A) Diagram (top) and x-ray image (bottom) taken with micro-CT showing the right hemisphere of mouse brain that radiation was delivered to. B) Normalized PET images with representative regions of interest drawn to show the irradiated (yellow) and non-irradiated (blue) brain hemispheres. C) Autoradiography image (left) and diagram (right) showing how the tissue samples were positioned on the slide. D) Histology images for irradiated and unirradiated hemispheres, with DAPI (blue) staining for cell nuclei and PBR (green) staining for TSPO.

Macrophage-Modulating Polymers for Local Delivery of Cancer Immunotherapeutics – Michael Celejewski

Background. Despite the potential of current and emerging cancer immunotherapies, efficacy is often hindered for many solid tumors, such as glioblastoma (GBM), due to transport barriers and therapy suppressive tumor-associated macrophages (TAMs). Drug delivery systems (DDS) implanted into tumor resection cavities have the potential to both overcome transport barriers and alter TAM bioactivities from therapy suppressive to supportive (Figure 1a, 1b).

Hydrogels are effective for localized drug delivery, as they overcome transport barriers by promoting sustained delivery of therapeutics at the tumor site. Moreover, hydrogels can be composed of biomaterials that promote modulation of tumor environments by increasing the ratio of pro-inflammatory (M1) to anti-inflammatory (M2) macrophages, as M2-like TAMs promote tumor progression and therapeutic resistance. Recent work has demonstrated that the RP-182 peptide (KFRKAFKRFF) can modulate TAMs by binding the cell surface protein CD206, triggering both selective M2-like TAM apoptotic depletion and repolarization towards M1-like TAMs.

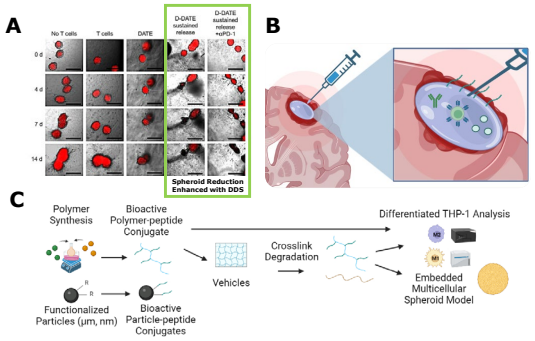

Figure 1: A. Previous work illustrating the impact of local delivery on spheroid cancer model outcomes, marked by a decrease in normalized spheroid area compared to no vehicle (adapted from Huynh et al., J Control Release, 2022). **B.** Therapeutic application of interest, implantation into tumor resection cavities for GBM, created by author with BioRender.com. **C.** Project experimental schematic created by author with BioRender.com. The polymer-based hydrogel platform will be formed and evaluated, while particle conjugates will be assessed for feasibility of increasing peptide density and adapted accordingly.

This work entails the design of immunomodulatory DDS composed of bioactive polymers that alter local TAM activity to improve local delivery for cancer drugs, such as adoptive cell therapies, bi-specific antibodies, and chemotherapeutics (Figure 1c). Hydrogels composed of polymers incorporating RP-182 for TAM depletion and repolarization, while demonstrating tunable degradation kinetics, will be developed for applications in local immunotherapeutic delivery. Particle-RP-182 conjugates will also be explored and evaluated in terms of their ability to control surface density of RP-182 for binding to TAM CD206 receptors.

Hypothesis. It is hypothesized that *RP-182 conjugated DDS will localize cancer drug release, and selectively modulate M2-like TAMs via depletion and repolarization*, to improve DDS for therapeutics hindered by M2-like TAMs and allow for increased therapeutic efficacy.

Methodology. *i) Conjugate synthesis and assessment.* A series of bio-inert poly(carboxybetaine-dibenzocyclooctyne N-hydroxysuccinimide) (pCB-DBCO) copolymers are being synthesized and conjugated to an azide-modified RP-182 peptide. Polarized THP-1 monocyte derived macrophages will be used to evaluate potential TAM modulation. Depletion will be tracked using cell viability assays with alamarBlue fluorescence, while repolarization capabilities will be tracked by flow cytometry with M1 marker CD86 and M2 marker CD206, as well as cytokine quantification.

ii) Gel formation and assessment. For tunable hydrogel degradation, a pCB-azide polymer with tunable hydrolytic carbamate bonds, promoted with electron withdrawing groups will be synthesized. The peptide-polymer conjugate will crosslink with pCB-azide to form the hydrogel. Bioactivity experiments analogous to conjugate studies will be performed for fully degraded hydrogel components.

iii) 3D cell-culture model. TAM-modulating DDS will be screened with embedded multicellular spheroids in the presence and absence of cancer therapeutics. The model will consist of the TAM-modulating hydrogel below a collagen hydrogel incorporating spheroids of cancer cells and TAMs. Spheroid size and cytokine levels will be tracked to evaluate TAM activities.

Impact. Injectable macrophage-modulating DDS amenable to localized drug release will aid in the modulation of tumor microenvironments from therapy suppressive to supportive, improving local drug delivery and immunotherapeutic efficacy for solid tumors.

Diffusion Model-Driven Radiomic Prediction of Gene Mutation Status Based On Magnetic Resonance Images of Breast Cancer

Lianghong Chen[1], Zi Huai Huang[2], Qian Liu[2], Pingzhao Hu[1,2,3,4],.

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[2]Department of Biochemistry, Western University
[3]Department of Epidemiology and Biostatistics, Western University
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Introduction: Breast cancer is a heterogeneous disease. It is crucial to analyze both multi-omic information and the tumour's phenotypic heterogeneity for breast cancer early detection and making personalized treatment options. Radiogenomics is an emerging field that studies medical images and multi-omic information. However, most radiogenomics studies face the challenge of missing either imaging data, genomic data, or clinical outcome data. We hypothesize that a well-trained deep diffusion model can generate breast cancer magnetic resonance images (MRI) based on patients' multi-omic profiles to address the unpaired data problem in the radiogenomics analysis of breast cancer. The generated MRI can be used to predict the mutation status of driver genes in breast cancer.

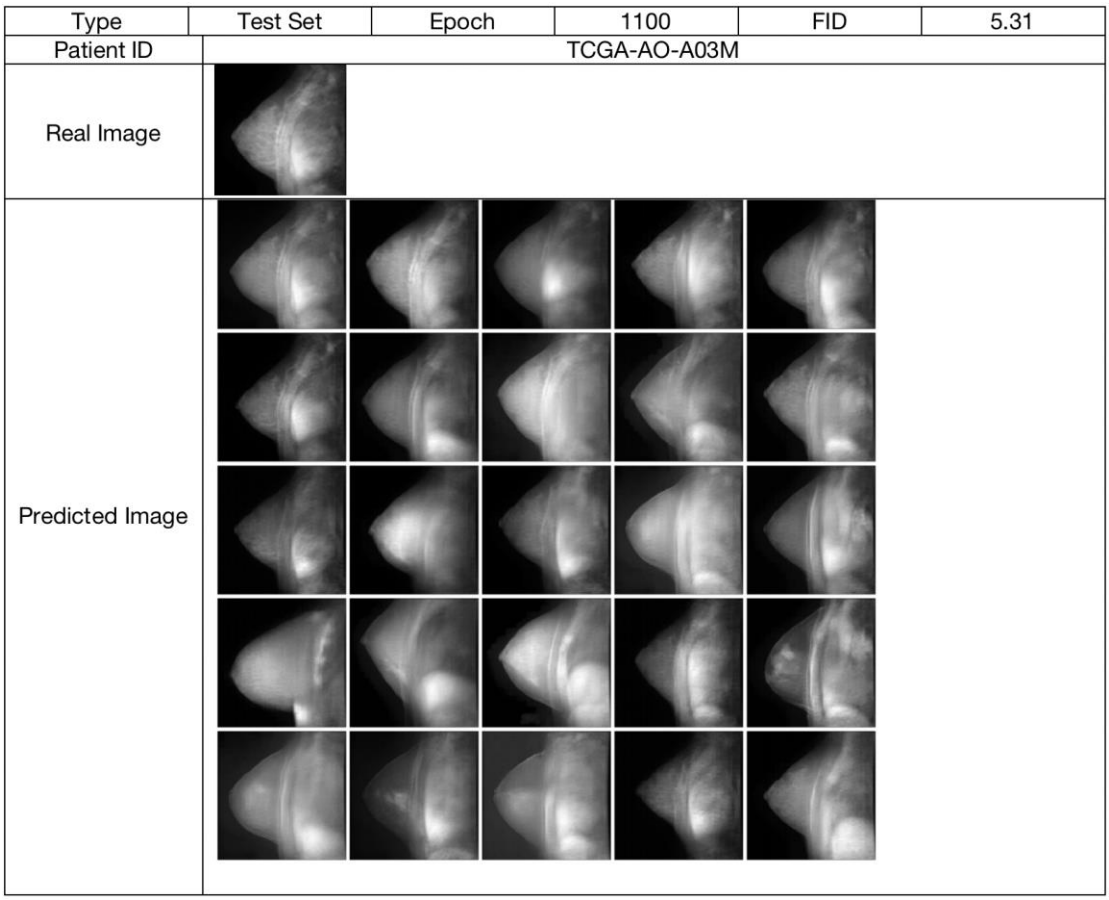
Methods: We use the paired sagittal MRI and multi-omic (RNA gene expression, DNA methylation, and copy number variation) profiles of 58 breast cancer samples from The Cancer Imaging Archive (TCIA) and The Cancer Genome Atlas (TCGA) to train a deep learning-based diffusion model, in which the multi-omic data is resolved to latent factors by Bayesian tensor factorization (BTF) as the condition to guide MRI generation. Model performance is measured by Frechet's Inception Distance (FID) which compares the quality of the MRI generated by the diffusion model and their corresponding real images. The well-trained model is then used to generate the MRIs based on a given patient's multi-omic latent features. The generated images are further used to predict the mutation status of BRCA1 and TP53 genes in 754 breast cancer patients using a convolutional neural network (CNN).

Results: We trained the diffusion model that is conditioned on the 17 latent multi-omic BTF features using 54 samples with the matched MRI and multi-omic data. The overall FID of the trained model based on the test set with 4 samples is 26.45. **Figure 1** shows the example images generated for the patient with ID TCGA-AO-A03M. Using the trained model, we generated the MRI for the 754 patients with only multi-omic profiles but no MRI data. We will train CNN models based on the generated MRI to predict the mutation status of gene BRCA1 and TP53, respectively (on-going).

Conclusion: These findings solidify the deep diffusion model as a potential tool to generate synthetic breast cancer MRI. It also lays the foundation for future breast cancer-related machine learning studies as the generated images can significantly augment the existing MRI data and avoid privacy issues for data sharing.

Keywords: Breast Cancer, Deep Learning, Diffusion Model, Mutation status, Radiogenomics, Magnetic Resonance Images

Figure 1: An example of a real image and the generated MRI conditioned on the multiomics profiles.



Interference with IL-18 proinflammatory signaling in human papillomavirus-positive head and neck cancers.

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The incidence of human papillomavirus-positive (HPV+) head and neck squamous cell carcinoma (HNSCC) is increasing at epidemic rates. Despite generally favourable treatment outcomes for HPV+ HNSCC compared to HPV-negative HNSCC, roughly 30% of patients still succumb to the disease. Additionally, many survivors experience lifelong treatment-related sequelae. Improved understanding of the effects of HPV in HNSCC is required for the development of effective therapies with fewer adverse consequences. HPV+ HNSCCs retain expression of the HPV E6 and E7 oncoproteins, which are critical for tumor growth, survival, and avoidance of the immune system. To aid in viral immune evasion, E7 can disrupt the methylation of several host gene promoters that interfere with HPV infection. One potential target of E7 is IL-18, a pro-inflammatory cytokine with a critically important role in epithelial barrier function. IL-18 activity is antagonized by the soluble IL-18 binding proteins (IL-18BP). Here we demonstrate the effects of HPV E7 on both IL-18BP and IL-18 expression in HNSCC. Analysis of RNA-sequencing data from the HNSCC cohort of The Cancer Genome Atlas shows increased *IL18BP* and reduced *IL18* mRNA levels in HPV+ HNSCC compared to HPV- HNSCC and normal adjacent tissues. This suggests a concerted antagonism of E7 on the IL-18 pathway. Higher levels of methylation also exist at CpG sites in the upstream region of *IL18* in HPV+ HNSCC. These observations indicate a possible mechanism for transcriptional repression of *IL18* in HPV+ HNSCC. Luciferase reporter assays demonstrate that HPV16 E7 expression alone can repress IL-18 transcription *in vitro*. Future experiments will establish a mouse model to test the therapeutic potential of restoring IL-18 signaling in HPV+ HNSCC. Our data regarding the effects of HPV and its E7 oncoprotein on IL-18 transcription provide insight towards the effect of this immune evasion mechanism on the host-pathogen relationship and the immune response within HPV+ HNSCC tumours. This insight provides valuable information for the development of future therapies for targeted reactivation of the immune response to HPV+ HNSCC.

Investigating the Gut Microbial Properties of Immunotherapy Responders and their Ability to Modulate Immune Responses in New Hosts using a DNA Mismatch Repair Deficient Neuroblastoma Model

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A growing line of evidence suggests a role for the gut microbiota as a modulator in anti-tumour immunity and improving the efficacy of immunotherapy treatments. Recent clinical trials have shown that transferring the microbiome of an immunotherapy responder via fecal microbiota transplant (FMT) to another patient with immunotherapy-refractory melanoma can sensitize 30% of those patients' tumours to anti-PD1 therapy [1,2]. However, the microbial features that are responsible for the therapeutic effects of microbiome transplants from responders to a new host remain elusive. Our lab has previously shown that inducing mismatch repair deficiency in neuroblastoma tumour-bearing mice cured 70% of these animals when treated with anti-CTLA-4 therapy [3]. However, these induced mismatch repair deficient (idMMR) neuroblastoma tumour-bearing mice remained refractory to anti-PD1 treatment. Therefore, this study aims to provide a mechanistic insight into the specific bacterial species or strains in the gut of immunotherapy-responsive mice that can transform the immune phenotype of tumours in new hosts and sensitize them to immunotherapy.

Flow cytometry was used to assess the immunomodulatory effects of FMT from anti-CTLA4-responsive- and isotype control-treated idMMR neuro-2a (neuroblastoma) primed mice on the immune phenotype of tumours in new hosts. To better understand the gut microbial composition of immunotherapy responders, the stool of anti-CTLA-4-responsive- and isotype control-treated idMMR neuro-2a primed mice were collected for downstream sequencing. Samples underwent DNA extraction, and region V4 the 16S rRNA gene was PCR amplified using established GOLAY barcoded primers. Amplicon libraries were processed at Robarts Research Institute and were sequenced on the MiSeq paired-end Illumina platform. The DADA2 (v1.8) pipeline will be used to process, align, and categorize to identify the exact sequence amplicon sequence variants (ASVs). Downstream analyses on the ASVs will be completed to infer the microbial profiles of the anti-CTLA-4-responsive animals. To determine the effect of bacteria in the stool of anti-CTLA-4-responders on the maturation of dendritic cells (DCs), the most predominant culturable bacterial strains will be grown on selective bacteriological media in an anaerobic chamber. Isolates will be co-cultured with bone marrow-derived DCs and the upregulation of DC maturation markers will be assessed by flow cytometry. To determine the ability of the predominant bacterial strains in sensitizing idMMR

neuro-2a primed mice to anti-PD1-therapy, the immune profiles of the tumours will be analyzed using flow cytometry, and tumour volumes will be measured.

Preliminary data suggests that the neuroblastoma tumours of mice that received an FMT from anti-CTLA-4-responsive mice had lower levels of overall tumour-infiltrating lymphocytes (TILs). However, the TILs of these FMT-treated animals were more tumour-specific, marked by an increase in CD39 expression, and had a higher level of activation marked by CD38 expression.

The preliminary results of this study currently reveal that FMT using stool from anti-CTLA-4-responsive mice can change the immune profile of neuroblastoma tumours in new hosts. Data generated in this study will provide mechanistic insights into the bacterial strains that possess immunomodulatory properties, which is of significant value in the future of cancer immunotherapies involving FMTs.

Keywords: Gut Microbiota, Immunotherapy, Immune Checkpoint Inhibitors, Cancer, Fecal Microbiota Transplant

References:

[1] Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin J-M, Morrison RM, et al. Fecal microbiota transplant overcomes resistance to anti–PD-1 therapy in melanoma patients. Science (1979) 2021;371:595–602. https://doi.org/10.1126/science.abf3363.

[2] Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science (1979) 2021;371:602–9. https://doi.org/10.1126/science.abb5920.

[3] El-Hajjar M, Gerhardt L, Hong MMY, Krishnamoorthy M, Figueredo R, Zheng X, et al. Inducing mismatch repair deficiency sensitizes immune-cold neuroblastoma to anti-CTLA4 and generates broad anti-tumor immune memory. Molecular Therapy 2022. https://doi.org/10.1016/j.ymthe.2022.08.025.

Regulation of natural killer cell responses by myeloid-derived suppressor cells in mouse mammary tumors

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Natural killer (NK) cells of the innate immune system play important roles in anti-cancer immunity. NK cell functions are regulated by inhibitory and activating receptors, which recognize specific ligands on the target cells. One example is the inhibitory NKR-P1B receptor which recognizes the ligand C-type lectin-related protein b (Clr-b). Previously, our lab has shown that tumor-infiltrating NK cells become dysfunctional rapidly in NKR-P1B-deficient mice compared to wild-type (WT) mice, suggesting a role for NKR-P1B:Clr-b interactions in NK cell functional homeostasis. The cellular interactions and factors involved in the induction of NK cell dysfunction in the tumor microenvironment (TME) are not fully understood. This project aims to understand the role of myeloid-derived suppressor cells (MDSC) in regulating NK cell responses via the NKR-P1B:Clr-b axis in TME. Using MMTV-PyVT transgenic mouse model of breast cancer and E0771 mammary adenocarcinoma cells injected into wild-type (WT), NKR-P1B-deficient, and Clr-b-deficient mice, we will assess MDSC recruitment and function in mammary tumors, and their effects on anti-cancer immune responses by NK cells. We have found that MDSCs express Clr-b, suggesting that they could potentially interact with NK cells via the inhibitory NKR-P1B receptor. While MDSC recruitment into the tumors in not affected, Clr-b expression is increased in MDSCs from NKR-P1B-deficient mice compared to WT mice. In *in vitro* co-culture assays, MDSCs inhibited IFNγ production by NK cells. MDSCs also induced downregulation of transcription factor EOMES and upregulation of checkpoint receptors Lag3 and PD-1 in NK cells, suggesting MDSCs can induce NK cell dysfunctions. Future experiments in trans-well plates and using inhibitors of known mechanism of immune suppression by MDSCs will explore the mechanism and the contribution of NKR-P1B:Clr-b interactions in immune suppression and NK cell dysfunction induced by MDSCs.

Assessing the Presence of Bacterial Cas9 in MLH1-knockout Neuroblastoma Cells

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Introduction: CRISPR-Cas9 is a prokaryotic adaptive immunity mechanism that has been adapted by scientists into a revolutionary gene editing tool. Previous work in our lab has used this process to knockout MLH1, a primary mismatch repair gene, in neuro-2a neuroblastoma cells to study whether these cells would then be sensitized to the immune system due to the formation of neoantigens. Immune monitoring of these neuroblastoma tumours indicated that the MLH1 knockout (KO) neuroblastoma cells were more immunogenic and had higher infiltration of T-cells. Nevertheless, there is a possibility that unintended Cas9 plasmid insertion and expression in these cells was the underlying cause of this increased immunogenicity, leading to confounding immune sensitization due to foreign bacterial protein presence. This study aims to assess the presence of bacterial Cas9 protein and transcripts from the CRISPR-Cas9 system in the MLH1-KO neuro-2a clones.

Methods: To detect Cas9 protein presence, we isolated protein from neuro-2a cells and ran a Western Blot to stain for Cas9 protein. To detect Cas9 mRNA presence, we isolated RNA from neuro-2a cells, generated cDNA from the RNA, and amplified this cDNA using PCR. Then, we performed DNA gel electrophoresis to identify Cas9 DNA presence.

Results: Our results demonstrate that neither Cas9 protein nor transcripts are present in our MLH1-KO cells.

Discussion: These findings provide evidence that Cas9 proteins and transcripts were not present in our MLH1-KO neuroblastoma cells. Therefore, Cas9 immunogenicity was not an interfering factor in the MLH1-KO cells, reinforcing previous findings of increased immune involvement due to neoantigen formation.

Keywords: CRISPR-Cas9, Neuroblastoma, Mismatch Repair, MLH1, Western Blot, RT-PCR

Poster #14

Title: **Immunotherapy treatment in colitis-associated cancer**

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Introduction:

Colorectal cancer is the second leading cause of cancer death in Canada. A major risk factor for the development of colorectal cancer is chronic inflammation leading to colitis-associated cancer (CAC). Recent clinical data suggests that the immune checkpoint inhibitor, a-PD-1, can induce a complete response in 100% of patients with mismatch repair-deficient locally advanced rectal cancer. However, treatment with anti-PD-1 of patients with CAC has not been explored. For this reason, we investigated the effect of anti-PD-1 in two different mouse models of CAC, including the AOM/DSS model and our Dclk1-CreERT2;APC^{ff} model. In the Dclk1-CreERT2;APC^{ff} model, colonic tumors arise from DCLK1+ cells following loss of the tumor suppressor adenomatous polyposis coli (APC) and induction of colitis.

Methods:

CAC was induced in C57Bl/6J mice by administration of a single dose of 10 mg/kg azoxymethane (AOM) followed by 2.5% dextran sodium sulfate (DSS) for 5 days. 2 weeks post DSS, mice were treated with 250 ug doses of either IgG isotype control antibody or anti-PD-1 given every 3 days x 3 doses. Twelve weeks post DSS, the mice were sacrificed and tumor number and size quantified. Tumor and adjacent non-tumor tissue were collected and protein isolated for analysis using the Eve Technologies Mouse High Sensitivity T-cell 18-Plex Discovery Assay. In separate experiments, we treated Dclk1-CreERT2;APC^{ff} mice with 3 doses of tamoxifen followed by 2.5% DSS. 2 weeks post DSS, mice were treated with 250 ug dose of IgG control antibody or anti-PD-1 given every 3 days x 3 doses. Fourteen weeks post DSS, mice were sacrificed and tumor number and size analyzed.

Results:

Using the AOM/DSS model of CAC, anti-PD-1 did not affect either tumor number or size compared to IgG treated mice. Similar observations were made using the Dclk1/Apcff model with no significant change in tumor number or size between IgG and anti-PD-1 treated mice. From adjacent non-tumor tissue of AOM/DSS treated mice, we detected a significant increase in the macrophage-derived cytokines TNFa and MCP-1. In addition, the neutrophil-derived cytokine KC was increased in mice treated anti-PD-1 versus IgG treated mice. We did not detect any differences in the cytokines within tumors between IgG and anti-PD-1 treated mice.

Conclusions:

Our findings demonstrate that anti-PD-1 was not effective in reducing tumorigenesis in colitis-associated cancer.

Impact of adjunct therapy with *Akkermansia muciniphila* in combination with anti-PD1 immunotherapy on a murine pancreatic cancer model

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Pancreatic adenocarcinoma (PDAC) is becoming a common cause of cancer mortality in Canada and around the world, with approximately only 10% of patients surviving 5 years past diagnosis. Due to the poor long-term outcomes and limited treatment options for PDAC, establishing new treatments is necessary to improve the survival of PDAC patients. Recently, the gut microbiome has been shown to play an important role in the body's response to tumors by influencing the immune system. Bacterial-based adjunct therapy has the potential to transform the TME to sensitize cancers to immunotherapy when present in the gut. *Akkermansia muciniphila* is one of the bacterial species that is routinely shown to be enriched in successful immunotherapy responders when present in the gut. *Akkermansia muciniphila* enhances anti-tumour immunity by transforming PDAC into immune hot tumors by increasing the recruitment of CCR9+CXCR3+CD4+ T cells and stimulating dendritic cells to promote IL-12 secretion. This study aims to investigate the effect of *A. muciniphila,* in modifying the gut- and tumour-microbiome and activating T-cells in PDAC. Additionally, we will determine whether the combination of bacterial-based therapy with anti-PD1 treatment can sensitize immunotherapy-refractory PDAC tumours to immunotherapy.

To determine the effects of *A. muciniphila* on PDAC tumour-bearing mice, $1\text{^}10^8$ colony forming units (CFU) of *A. muciniphila* or phosphate buffered saline (PBS) will be orally administered to $Ptf1a^{creERT/+}KRAS^{G12D}$ (PK) mice (n=10, 5 mice per group) with pancreatic tumors three times per week for two weeks. Stool will be collected pre- and post-treatment, and before the end of the experiment, to assess the changes in the gut microbiota by 16S rRNA gene sequencing. PDAC tumor tissues will also be harvested and will be profiled by 16S rRNA gene sequencing using low abundance microbiota methodology, as well as flow cytometry to determine changes in immune activation. To further understand the immune activation and tumour infiltration properties related to A. muciniphila, mixed lymphocyte reaction assays will be completed with dendritic cells from naïve mice and either treated with A. muciniphila or co-cultured with splenocytes harvested from tumor-bearing animals Finally, to examine whether oral bacterial therapy can sensitize PDAC tumours to immunotherapy with anti-PD1, PK tumor-bearing male and female mice (n=20, 5 mice per group) will be treated with the *A. muciniphila* or PBS in combination with either anti-PD1 immunotherapy or an isotype control antibody injection. Two weeks following the completion of the treatment, tumours will be excised and weighed to assess tumour burden.

As the proportion of PDAC mortality continues to increase in Canada and around the world, new therapeutic interventions are imperative for improving patient survival and quality of life. This study will provide a better understanding of the link between the gut microbiome and cancer therapeutics and will test novel combination therapy for the treatment of pancreatic cancer that is otherwise untreatable with current regimens.

Keywords: Gut Microbiota, Immunotherapy, Immune Checkpoint Inhibitors, Cancer, Akkermansia muciniphila

THE ROLE OF ACTIVATION-INDUCED CYTIDINE DEAMINASE IN B CELL ACUTE LYMPHOBLASTIC LEUKEMIA.

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Activation-Induced Cytidine Deaminase (AID, encoded by *Aicda*) plays a primary role in mutating cytidines during somatic hypermutation and forming DNA breaks during class switch recombination, producing high-affinity antibodies and B cell memory. Most AID mutations target immunoglobin genes; however, AID may mutate off-target genes creating oncogenic alterations, leading to B cell acute lymphoblastic leukemia (B-ALL). Our laboratory previously generated the Mb1-CreΔPB (B cell-specific PU.1/Spi-B knockout) mouse model to explore the role of PU.1 and Spi-B in B cell maturation. These mice develop B-ALL at 100% incidence by 18 weeks of age. Whole-exome sequencing of leukemias from these mice revealed C⟶T transition mutations in a trinucleotide context compatible with being induced by AID. Therefore, we hypothesize that PU.1/Spi-B function as negative regulators of *Aicda* during B cell development. We found that *Aicda* transcription was up-regulated in cultured leukemia cells lacking PU.1/Spi-B. Using ChIP-seq, two regulatory regions (R1 & R2) within the first intron of *Aicda* were identified to bind PU.1. CRISPR mutagenesis was used to disrupt predicted PU.1/Spi-B binding sites in the *Aicda* regulatory region in a cultured pre-B cell line. The clones are being stimulated with lipopolysaccharide (LPS) and analyzed with RT-qPCR to determine the effects of these mutations. Preliminary results showed that larger deletions in the R1 regulatory region resulted in up-regulation of *Aicda* gene expression in response to LPS stimulation. However, alteration of the PU.1/Spi-B binding sites in the *Aicda* gene resulted in no change in *Aicda* gene expression, which suggests that PU.1/Spi-B does not act in a silencer fashion. In the second phase of this project, the *Aicda* regulatory regions were cloned and ligated into a luciferase-reported vector to examine the repressive ability of PU.1/Spi-B using transient transfection assays. Initial results show that the R1 region assists in repression by the silencer element, however, mutation of the PU.1/Spi-B binding site in R1 had no impact on this repression. In future studies, ChIP-qPCR analysis will examine the impact of the mutations on PU.1 binding. These findings will contribute to a deeper understanding of the *Aicda* gene and the role of AID in pediatric B-ALL.

Multiplexed Immunofluorescence Imaging by Iterative Bleaching

Capturing the diverse phenotypes and interactions of immune cells in situ.

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Background: $CD4^+$ T cells are a heterogenous population which have been described to drive allergic disease. Tfh cells are the $CD4^+$ T cell subset that drive allergen-specific IgE antibody production, which causes the symptoms of allergic disease upon allergen ingestion. Effector $CD4^+$ Th2 cells are also generated upon allergic sensitization, which preferentially localize in peripheral tissues such as the lungs and intestines. However, as these cells have been shown not to contribute to IgE production, little is known about how they contribute to the disease manifestations of food allergy. This is in part because while current quantitative methods such as flow cytometry can identify unique subsets of $CD4^+$ T cells, they cannot capture their spatial localization and interactions with immune and stromal cells, meaning the cells targeted by Th2 cells have not been characterized. Such interactions may be visualized using traditional fluorescence microscopy; however, acquisition is limited to 3~8 parameters per tissue, which is insufficient to fully characterize heterogeneous cell populations involved in allergy.

Objective: We aimed to overcome this limitation by adapting and optimizing a recently developed multiplexed imaging technique for use in allergy research.

Results: We optimized and validated ~70 commercially available antibodies for use in immunofluorescence imaging of allergic tissues. To extend the multiplexity of allergic tissues, an iterative bleaching technique was employed. Using lithium borohydride to inactivate fluorophores, we acquired images of allergic lymph node and intestine (Fig. 1) tissues with >20 parameters by introducing new fluorophores cyclically. Major cell populations were identified by machine-learning cell segmentation, and single cell analyses and phenotype clustering analyses were performed. Closely related and frequently interacting immune and stromal populations were identified. Additionally, we developed a strategy to trace allergen-specific $CD4^+$ T cells by adoptively transferring ovalbumin-specific cells from OT-II mice bearing the congenic marker CD90.1 into hosts prior to challenge.

Discussion/Future Directions: The platform we have optimized will allow us to continue to explore heterogenous $CD4^+$ T cell populations within allergic intestines and make qualitative observations of intercellular relationships with potential relevance to therapeutic interventions. Additionally, we have and will continue to contribute to a worldwide database of validated commercially available antibodies for use in multiplexed immunofluorescence imaging. This work has broad implications for the field of immunology outside of allergy research for any field requiring deep phenotypic characterization.

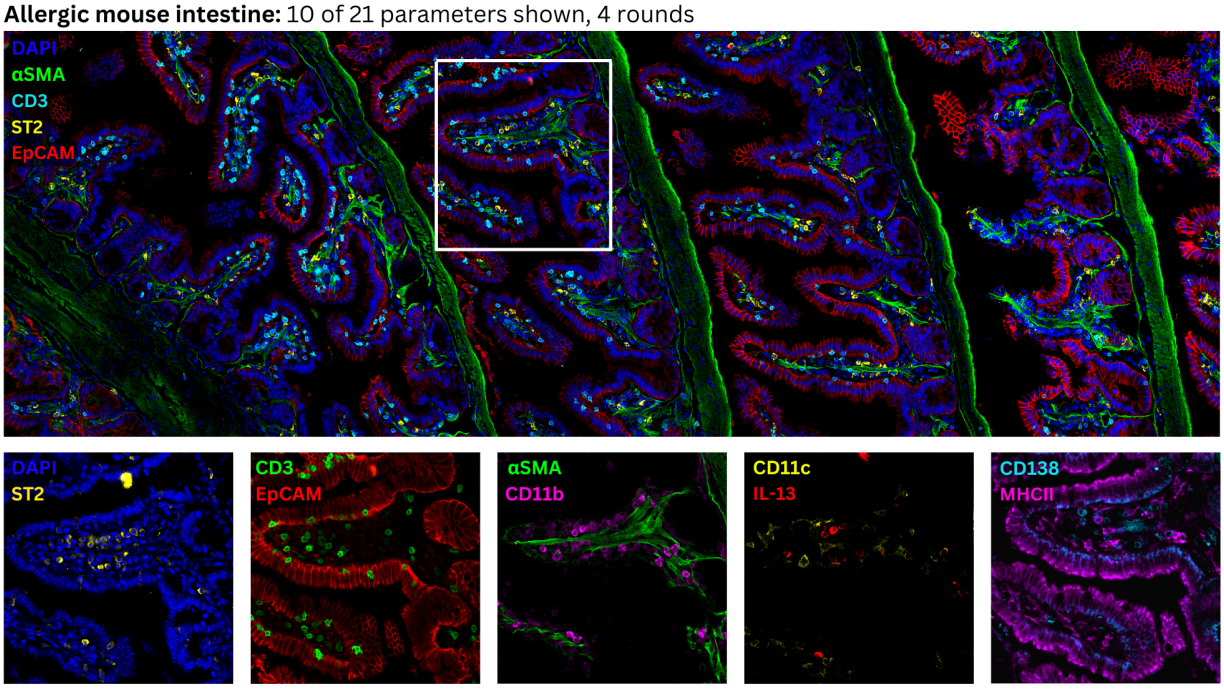

Fig. 1: Multiplexed confocal images of the intestine of a mouse that received antigen-specific T cells and was subsequently challenged with ovalbumin. Small intestines were obtained and processed into swiss rolls, fixed, frozen, and cryosectioned to best preserve epitopes. 4 rounds of imaging were conducted, each followed by treatment with lithium borohydride to inactivate fluorophores. Images feature the same section of tissue across rounds, aligned and registered for computational analysis. 21 immune and stromal parameters were imaged, allowing for the characterization of diverse cell subsets.

Effect of pharmacological inhibition of endogenous hydrogen sulfide production on high-grade bladder cancer progression

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Introduction: Current intravesical bladder cancer treatment paradigms have limited therapeutic impact requiring investigation of novel therapies. Recent evidence suggests hydrogen sulfide (H_2S), an endogenous signaling molecule, mediates cancer progression. The purpose of this study is to investigate the effect of inhibiting endogenous H_2S synthesis on bladder cancer progression with or without the chemotherapeutic drug, gemcitabine (GEM).

Methods: To first investigate this effect *in vitro*, MB49 cells are treated with the H_2S synthesis inhibitor, propargylglycine (PAG), the H_2S donor, sodium hydrosulfide (NaHS), and GEM and their combinations. Cell viability is analyzed using flow cytometry. Subsequently, an intravesical bladder cancer mouse model has been established using the carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine, which develops non-muscle invasive bladder cancer after 12 weeks of exposure. Mono and dual therapies are delivered via transurethral administration weekly for 4 weeks. Magnetic resonance imaging is utilized to detect cancer presence and monitor tumor burden and progression. These findings will be further validated by pathology which will characterize bladder cancer stage and grade. Immunohistochemistry will be utilized to investigate the immune response and other markers of bladder cancer progression.

Results: Our *in vitro* results show that compared to a control group, treated with saline, significant attenuation of bladder cancer cell survival was demonstrated by PAG ($p<0.001$) and GEM ($p<0.001$) but potentiated by NaHS ($p<0.001$; Figure 1). Further attenuation was demonstrated by PAG + GEM dual therapy, which was significantly less than the control ($p<0.001$) and PAG monotherapy ($p<0.01$) suggesting an additive effect. NaHS appears to abrogate the anti-cancer effects of PAG, partially recovering bladder cancer cell viability compared to PAG ($p>0.05$) suggesting the anti-cancer effects of PAG are due to decreased H_2S presence. Current *in vivo* findings demonstrate similar trends as PAG monotherapy demonstrates a noticeable reduction in tumor progression, which is partially recovered by NaHS, when compared to the control group.

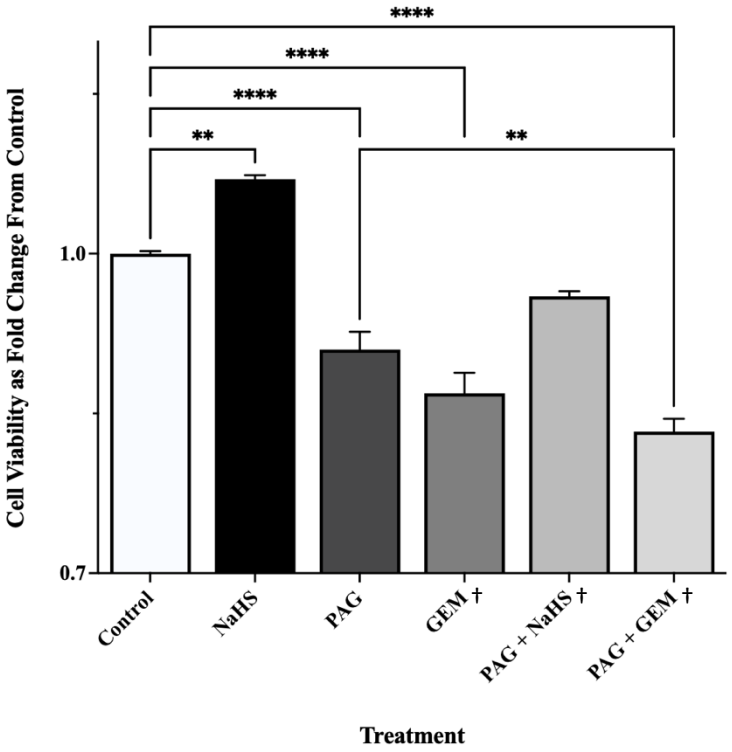

Figure 1. Analysis of MB49 cell viability, as fold change, with single and dual therapies (n = 5, †n = 3). Cells were stained with flow cytometry probes Annexin-V and propidium iodide. Data are expressed as mean ± standard error of the mean, **p<0.01, ****p<0.0001.

Conclusion: These findings provide evidence that suggests inhibiting H_2S synthesis hinders bladder cancer progression and enhances the anti-cancer effects of chemotherapy. This work will set the foundation for future clinical trials, which could lead to the inclusion of H_2S-targeted therapies into the armamentarium of urologic oncologists.

Immunostimulatory effects of secreted *Helicobacter pylori* proteins on gastric cells

Noor Salloum and Carole Creuzenet

Helicobacter pylori (HP) colonizes half of the world population, causing gastric inflammation that can lead to ulcers and cancers. A secreted immunostimulatory protein and its gate keeper are essential for the immunogenic response to HP. The immunostimulant's structure is mainly made of Sel-Like Repeats (SLR) motifs – maintaining the structure by disulfide bonds – are known to be important in protein-protein interactions that are involved in signal transduction across kingdoms. The gatekeep is key for disulfide bond formation in the immunostimulant and its secretion outside of the cell.

Our aim is to identify the effects of the immunostimulant and the gatekeeper and to identify a host interacting protein partner, using human adenocarcinoma gastric (AGS) cells. To assess their effects on the proinflammatory response, AGS cells were exposed to wild-type and knock-out HP mutant strains. IL8, a pro-inflammatory cytokine, was assessed by ELISA. Also, bacterial culture supernatants were used to avoid signaling from other bacterial translocated components. Immuno-Electron Microscopy (IEM) was used to demonstrate the localization of the immunostimulant in the host cell. Finally, to identify the host interacting partners, we will implement in-situ biotinylation of interacting partners by BioID, using a BirA fusion plasmids transfected in AGS cells. The biotinylated partner/s will be pulled down and analyzed by Mass spectrometry.

There was significant immunostimulant/gatekeeper-dependent stimulation of IL8 secretion when AGS cells were exposed to the different live HP strains. Using IEM, the immunostimulant was detected inside the host cells when exposed to wild-type HP. Furthermore, we have detected by Westernblot biotinylated protein/s as potential partner/s (to be analyzed by mass spectrometry). This suggests there might be some interaction between the immunostimulant and the host protein/s. The identification of interacting proteins will provide a better understanding of HcpE's mechanism of action and provide targets for inhibition that could be applied as novel therapeutics.

4th Annual Immuno-Oncology Symposium 2023 Abstract

Immuno-Imaging session

Efficient CRISPR genome editing of HER2-targeted CAR-T cells co-expressing multimodal imaging reporter genes.

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Introduction:
Adoptive transfer of T cells genetically engineered to express chimeric antigen receptors (CARs) has shown remarkable results in the treatment of numerous types of blood cancer. Despite the US approval of seven CAR-T therapies, this strategy still faces several limitations including an unpredictable response that varies by patient and the lack of clinical success in treating solid tumors[1]. In the past, the use of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) editing to target the *TRAC* locus and knock out the endogenous T cell receptor (TCR) showed improved CAR-T cell phenotype and tumor killing with increased survival in pre-clinical tumor models[2]. Based on this seminal paper, we developed a highly efficient system to generate TCR^- T cells co-expressing a CAR and preclinical or translationally relevant imaging reporter genes. Our imaging tools will allow a better approach to studying CAR-T trafficking, expansion, and tumor infiltration in pre-clinical cancer models and may eventually be applied to patients to understand individual treatment responses.

Methods:
CRISPR editing and adeno-associated virus (AAV) transduction were employed to knock in a human epidermal growth factor receptor 2 (HER2)-specific CAR[3] at the *TRAC* locus[2] of primary human T cells. Green fluorescent protein (eGFP), luciferase reporter AkaLuc[4], organic anion transporting polypeptide 1B3 (OATP1B3)[5] or human sodium iodide symporter (NIS) [6] genes were cloned downstream of the CAR in AAV donors to enable tracking by fluorescence, bioluminescence (BLI), magnetic resonance imaging (MRI), or positron emission tomography (PET), respectively. Flow cytometry and junction PCR were used to assess editing efficiency. *In vitro* BLI, and MRI contrast agent uptake assays were used to demonstrate the imaging suitability of the engineered CAR-T-reporter cells. *In vitro* cytotoxicity was assessed by BLI of firefly luciferase (Fluc)-expressing human SKOV3 (HER2+) ovarian cancer cells co-cultured with CAR T cells at different target: effector cell ratios. Tracking of CAR-AkaLuc T cell expansion and tumor homing was evaluated by BLI in Nod-SCID-gamma mice bearing subcutaneous SKOV3 tumors.

Results:
Overall, TCR knockout efficiency was ~89% with variable HER2CAR expression ranging from 40-80% HER2CAR$^+$ cells that was dependent on the reporter gene used. HER2CAR-T cells engineered with AkaLuc or OATP1B3 were functional *in vitro* where the BLI signal was proportional to the number of HER2CAR-AkaLuc cells and uptake of Gadolinium ethoxybenzyl-diethylenetriaminepentaacetic acid (Gd-EOB-DTPA) contrast agent trended 2.3 times higher in HER2CAR-OATP1B3 than in Naïve T cells. HER2CAR$^+$TCR$^-$ T cells killed co-cultured SKOV3-tdTomato-Fluc cancer cells significantly higher than naïve T cells ($p<0.001$). A single intravenous dose of $5x10^6$ HER2CAR-AkaLuc T cells at day 15 post-tumor implantation did not reduce SKOV3 subcutaneous tumor growth but elicited significant homing and expansion of HER2CAR-Akaluc T cells in subcutaneous tumors and the lungs of tumor-bearing mice by 14 days after intravenous infusion.

Conclusions:
Our strategy demonstrated an efficient CRISPR-editing system for CAR-T cells useful for non-invasive imaging with reporter genes and the CAR construct inserted at the *TRAC* locus. Our current work is focused on optimizing CAR-T cell detection by MRI and PET to enable better testing of CAR designs in preclinical models.

References:

1. Hong, M., Clubb, J. D. & Chen, Y. Y. Engineering CAR-T Cells for Next-Generation Cancer Therapy. *Cancer Cell* **38**, 473–488 (2020).
2. Eyquem, J. *et al.* Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumor rejection. *Nature* **543**, 113–117 (2017).
3. Hammill, J. A. *et al.* Designed ankyrin repeat proteins are effective targeting elements for chimeric antigen receptors. *J. Immunother. Cancer* **3**, 55 (2015).
4. Bozec, D. *et al.* Akaluc bioluminescence offer superior sensitivity to track in vivo glioma expansion. *Neuro-Oncol. Adv.* **2**, vdaa134 (2020).
5. Baek, S.-E. *et al.* Human Organic Anion Transporting Polypeptide 1B3 Applied as an MRI-Based Reporter Gene. *Korean J. Radiol.* **21**, 726–735 (2020).
6. Sakemura, R. *et al.* Dynamic Imaging of Chimeric Antigen Receptor T Cells with [18F]Tetrafluoroborate Positron Emission Tomography/Computed Tomography. *JoVE J. Vis. Exp.* e62334 (2022) doi:10.3791/62334.

Poster #21

Visualization of a HER2-specific CAR NK-92 cell immunotherapy with Bioluminescence Imaging

Nourhan Shalaby

Abstract:

Ovarian cancer is the fifth most common cause of female cancer deaths. This statistic emphasizes the need to develop new therapies that have the potential to improve the survival of women with this devastating disease. For decades the mainstay treatments for solid tumours were surgery, chemotherapy and/or radiotherapy. More recently, cell-based immunotherapies, administering immune cells that have been genetically modified outside the body to better target and kill cancer cells, has shown transformative and even curative clinical results, and is being massively explored for the treatment of a variety of malignancies. Despite their remarkable clinical success for the treatment of hematological malignancies, CAR therapies have significant hurdles that need to be overcome to broaden applicability to solid tumours. There is a clinical unmet need to track these cells in each patient and to better understand their in-vivo persistence and long-term survival.

In this work, Natural Killer (NK) cells have been genetically engineered to express a Chimeric Antigen Receptor (CAR). The CAR is designed to bind to a specific tumour-associated antigen present on the surface of ovarian cancer cells, called the human epidermal growth factor receptor 2 (HER2). NK cells were also engineered to express bioluminescence (BLI) and positron emission tomography (PET) reporter genes to allow for multi-modal imaging. Flow cytometry and lysis assays were performed to assess gene expression and functionality, respectively. Immunocompromised mice were inoculated with ovarian cancer cells. A week later, (n=6) mice received 3 injections of 15 million engineered CAR-NK cells, three days apart. A control group of mice (n=4) received non-engineered NK cells. Mice were given daily i.p. injections of 12,500 units of IL-2 to prolong NK in-vivo survival. Another control group of mice received sham injections of PBS (n=4). Tumour burden was monitored with BLI over time. NK cells were monitored with both BLI and PET.

Cells were FACSorted and collected with 97% purity. Mice receiving both naïve NK and PBS injections had continuous tumour growth as seen by BLI. Mice receiving the HER2-targetting CAR NK cells showed significant tumour suppression ($p<0.05$) on days 11, 14 and 18 when compared with control mice. Mice receiving the engineered NK therapy also exhibited increased overall survival, as demonstrated by the Kaplan-Meier survival curve.

In this work we report a dual BLI imaging of both tumour progression and HER2-CAR-NK therapy. We showed that the NK therapy was capable of tumour suppression and increased survival. This method also allowed the monitoring of therapy cells to better understand their localization, proliferation and long term in-vivo persistence.

The role of TEX-derived circHUWE1 in prostate cancer migration and metastasis

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Introduction: Immunotherapy has been an emerging area of cancer research; however, the clinical efficacy of immunotherapy varies greatly. Tumour-derived exosomes (TEXs) display enhanced expression of tumour antigens, and can be used in cancer treatment, either directly or by stimulating DCs for DC-based cancer vaccines. Circular RNAs (circRNAs) exert their functions by acting as microRNA (miRNA) sponges or altering parental gene translation. We have previously shown that circHUWE1 is differentially expressed in highly metastatic (Met-high) prostate cancer (PCa) cells compared to low metastatic (Met-low) PCa cells. In the present study, we attempt to assess the role of TEXs in tumour metastasis, and elucidate the mechanisms by which circHUWE1 may modulate prostatic cancer pathogenesis. We hypothesize that circRNA in TEXs promotes tumour cell proliferation, migration, and invasion. Therefore, manipulating TEX-derived circRNA (ex. circHUWE1), should reduce tumour cell proliferation/migration/invasion, and metastasis.

Methods: Met-high (PC3) and Met-low (LNCaP) cells were cultured in RMPI-1640 medium supplemented with 10% fetal bovine serum at 37°C, 5% CO2. PCa cells were transfected with circHUWE1 siRNA, HUWE1 siRNA, and C-MYC siRNA using Endofectin transfection reagent. Exosomes from PC3 and LNCaP cells were isolated using ultracentrifugation. Specifically, PCa cells were cultured in complete culture medium until approximately 80% confluent, then medium was replaced with serum-free medium for 24 hours to ensure isolated exosomes were from the cells of interest. The medium was then collected and centrifuged (300g, 4°C, 10 mins) to remove any detached cells. The supernatant was filtered using a 0.22μm filter to remove cell debris, apoptotic bodies, and microvesicles. The filtered supernatant then underwent two ultracentrifugation steps (100,000g, 4°C, 90 mins) to pellet the exosomes. Exosomes were then characterized by detection of an exosomal marker, CD63, using Western blot. The cellular and exosomal expressions of circHUWE1, HUWE1, and C-MYC were analyzed using qPCR and Western Blot. Cell migration was assessed using a scratch assay.

Results: Knockdown of circHUWE1, HUWE1, and C-MYC in PC3 cells using siRNA transfection significantly reduced the mRNA levels compared to control, as confirmed by qPCR. Exosomes were successfully isolated from both serum-free and normal cell culture supernatant of PCa cells, as confirmed by the presence of CD63 in the exosome protein samples. 600ng of exosomal RNA was isolated from 50mL culture medium from approximately 30 million PC3 cells. 150μg of exosomal protein was isolated from 50mL culture medium from approximately 30 million PC3 cells. mRNA expression of circHUWE1, HUWE1, and C-MYC were detected in both the PC3 cells and exosomes. Preliminary results from the scratch assay showed transfection

with HUWE1 siRNA and C-MYC siRNA inhibited migration of PC3 cells compared to untransfected controls.

Discussion: Understanding the contribution and mechanism of circHUWE1 in TEXs to Met-low/high PCa progression may allow for the development of targeted therapeutics. Modulated TEXs may be used to prevent tumour metastasis and improve anti-cancer therapy.

Abstract

Title

Analysis of Variables Predicting Pathological Complete Response and Immune related adverse events in Patients with Resectable Non-Small Cell Lung Cancer receiving Neoadjuvant Immunotherapy with Chemotherapy – A Prospective Cohort Study ('Pre-PLaN')

Authors

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Background: Neoadjuvant (NA) chemo-immunotherapy (C-IO) has shown a better event-free survival (EFS) and markedly increased pathological complete responses (pCR), vs chemo-alone, in resectable non-small cell lung cancer (NSCLC) in CheckMate 816 trial. It showed that achievement of pCR resulted in markedly prolonged EFS. This approach is becoming the new SoC. Given the importance of pCRs, there is an increasing interest in predicting its occurrence. Also, around one in three patients has grade 3 immune related adverse events (irAEs) with this approach. The response as well as toxicity are related to interplay between host's immunity and tumor variables. We propose to analyze the predictors of pCR and irAEs with NA C-IO in resectable NSCLC, employing a range of wider and more sophisticated parameters that reflect both the tumor and the integrity of host's immune system. We will then seek to develop an initial integrated predictive model for pCR/non-pCR, and irAEs, with a good sensitivity/specificity, as a prelude to testing (and if necessary, refining) the model in future.

Methods:

This is single center, prospective cohort study enrolling patients with stage IB-IIIA NSCLC, ECOG-PS 0-2 without EGFR, ALK or ROS1 mutations. Patients will be treated with NA nivolumab plus

platinum doublet chemotherapy. The planned accrual is for 60 patients. The primary hypothesis is that a model combining predictive variables for pCR in patients with resectable NSCLC treated with NA C-IO can predict a pCR with an AUC of at least 0.8. The primary objectives are to explore the feasibility of acquiring a combination of baseline and treatment-emergent potentially predictive variables (table 1); to determine the predictive power of these variables; and to develop a precise model to predict pCR and irAEs. The secondary objectives are to assess pCR, major pathological response (MPR), objective response rates (ORRs), and irAEs; to evaluate exploratory and entirely novel potential biomarkers for predicting responses and irAEs and to assess if a post-treatment (pre-surgery) complete metabolic response by F18-FDG-PET-CT and a blood-only molecular residual disease assay can accurately predict a pCR.

Table 1 - Baseline and treatment-emergent potentially predictive variables

Demographic	Clinical	Radiology	Blood-based	Pathology
			Flow (cell subsets)	
			Immune activation markers	Histology
			LDH and its isoenzymes	PDL1
Age	Co-morbidities			Mulitiplex IHC for TME cell types and proteins (LAG3, LSD1, and CD47)
Gender	Medications	Body composition	CEA	
Ethnicity	ECOG PS	CT scan	CA199	
Smoking status	Weight loss	SUV (PET/CT)	CA125	NGS (TMB)
Vaccination	Stage	Radiomics	LIF	Spatial transcriptomics
COVID status	BMI		CRP	pCR MPR
			IL6	
			IL7	Fecal and tumor microbiome
			GDF-15	
			ctDNA clearance	

Prolonged remission of metastatic cisplatin-refractory nasopharyngeal carcinoma with pembrolizumab.

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Background: Epstein-Barr virus (EBV)-related nasopharygeal cancer is a common type of cancer in certain areas of the world such as southeast Asia, but is uncommon in Canada. For patients with incurable metastatic EBV-related nasopharyngeal cancer that progresses after first-line therapy with gemcitabine/cisplatin, there is currently no reliably effective standard treatment.

Patient and methods: With his consent, the health records of a patient with relapsed metastatic EBV-related nasopharyngeal cancer treated with pembrolizumab immunotherapy were retrospectively reviewed and reported.

Case report: A male patient presented at age 15 with stage IVA EBV-related nasopharyngeal cancer. Despite response to initial chemoradiation and adjuvant chemotherapy, the patient experienced metastatic cancer relapse in lymph nodes and bone. There was initial response to gemcitabine/cisplatin chemotherapy, but signs and symptoms of progressive cancer appeared after 7 cycles. The patient was then switched to pembrolizumab 200 mg IV q3weeks, funded through the LRCP ACTNOW program, and he had a near complete clinical response after 14 cycles. Serum EBV titres have normalized and CT imaging shows only some healed bone metastasis. Retrospective assessment of tumor CPS PD-L1 was >20. Hypothyroidism developed, possibly due to radiation treatment, but otherwise he did not experience any other immune-mediated toxicities on or following treatment, which lasted in total 2 years with 41 cycles. To date, the patient has been observed off pembrolizumab for over one year and is highly functional and without evidence of disease progression.
Conclusions: This case illustrates the potential benefit of immunotherapy for improving survival and quality of life in selected patients with metastatic EBV-positive cisplatin-refractory nasopharygeal cancer.

Visualizing natural killer cell communication using activatable magnetic resonance imaging

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INTRODUCTION: In recent years, natural killer (NK) cells have garnered increased attention as potential sources for cell-based immunotherapies to combat cancer, presenting safety and production advantages over T cells[1]. To improve the development and translation of these NK therapies, imaging tools capable of non-invasively visualizing how NK cells interact with their intended targets in the body would be highly valuable. We describe a non-invasive tool to visualize when immune cells interact with its targeted antigen on cancer. The synthetic Notch (SynNotch) receptor is a versatile system that signals antigen-specific cell-interaction via activated transcriptional modulating of any transgene[2]. Our goal was to engineer NK cells that will activate imaging reporter genes upon binding specific cancer antigens.

METHODS: We engineered a human natural killer (NK-92) cell line via lentiviral transduction of two components: (1) SynNotch receptor directed against the B-cell surface antigen CD19[3], and (2) a response element containing tdTomato (tdT) for fluorescence, firefly luciferase (FLuc) for bioluminescence (BLI), and organic anion transporting polypeptide 1B3 (OATP1B3) for MRI[4] (Fig. 1A-B). For our subcutaneous tumour model, NSG mice were implanted with either CD19+ or CD19- Nalm6 leukemia cells subcutaneously. Once tumours reached >100 mm^3, mice received an intratumoural injection of 10^7 irradiated engineered NK-92 cells. For our systemic model, mice received intravenous injection with $5x10^6$ CD19+ Nalm6 cells expressing the Antares BLI reporter, and once cancer cells were allowed to grow for 11 days, mice received an intravenous injection of $5x10^6$ irradiated engineered NK-92 cells. Reporter activation was assessed by FLuc BLI and gadolinium-enhanced T_1-weighted MRI.

RESULTS: BLI revealed minimal background in subcutaneous tumours prior to NK-92 cell injection (Fig. 1C). Excitingly, as early as 12 hours post-intratumoural cell injection, CD19+ tumours exhibited significantly higher FLuc signal than CD19- tumours. Following administration of the gadolinium agent Primovist, MRI revealed notable regions of contrast enhancement in CD19+ tumours, with significantly higher contrast-to-noise compared to similar regions in CD19- tumours (Fig. 1D). Following intravenous NK-92 injection, FLuc was near undetectable in healthy mice, whereas in tumour-bearing mice, FLuc signal was significantly higher in the midsection compared to healthy mice (Fig. 1E). Ex vivo BLI revealed significantly greater FLuc signal in the spleen of tumour-bearing versus healthy mice, as well as elevated FLuc in liver and lungs (Fig. 1F).

DISCUSSION: In this work, we have demonstrated the antigen-dependent activation of imaging reporter genes in NK-92 cells interacting with cancer cells– notably observing cell-cell interaction via a human-derived, clinically-relevant MR reporter for the first time *in vivo*. With further refinement, we posit these tools will improve our ability to understand and monitor cell-cell interactions in multicellular organisms, which could be broadly applicable to better understand development, homeostasis, and beyond.

REFERENCES: [1] Tang et al., *Am J Cancer Res*, 2018, 8: 1083-1089. [2] Morsut et al., *Cell*, 2016, 164: 780-791. [3] Sadelain et al., *J Clin Invest*, 2015, 125(9): 3392-3400. [4] Nystrom et al., *Invest Radiol*, 2019, 54(5): 302-311.

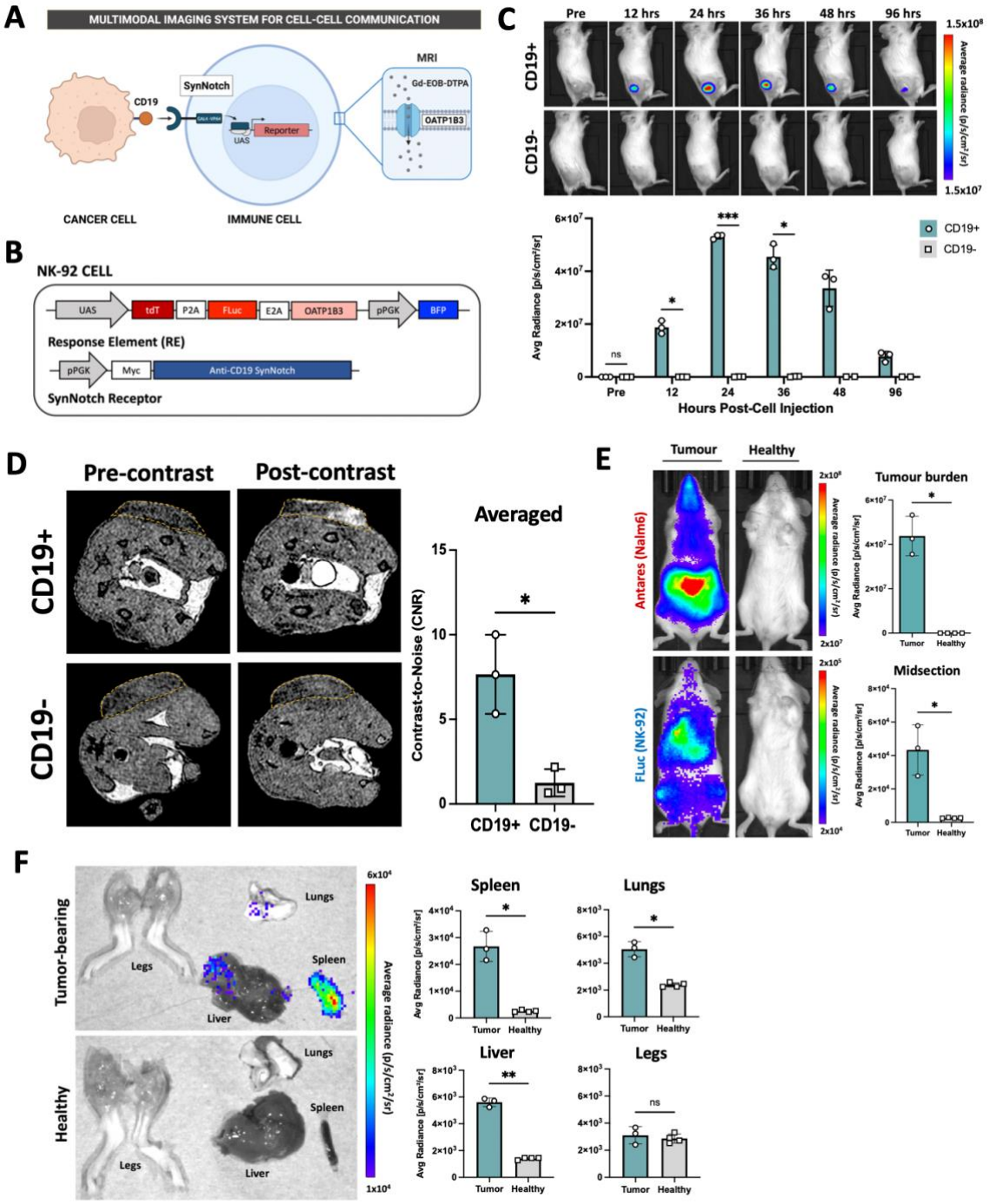

Figure 1. **Natural killer cells with SynNotch gene circuits exhibit antigen-dependent reporter activation**. **(A)** Schematic of SynNotch imaging system. CD19 binding by the CD19-targeted SynNotch induces translocation of a GAL4-VP64 transactivator. **(B)** Human NK-92 cells (Jurkats) were engineered with CD19-targeted SynNotch driven by the phosphoglycerate kinase 1 promoter (pPGK), and a response element containing trimodal reporter genes as well as a pPGK-driven blue fluorescence protein (BFP) for sorting. **(C)** FLuc BLI in mice with subcutaneous CD19+ or CD19- Nalm6 tumours pre- and post-intratumoral injection with irradiated engineered NK-92 cells (N=3). **(D)** T_1-weighted MR images acquired pre- and 5 hrs post-contrast. Tumours are outlined in yellow on representative transverse slices. Average contrast-to-noise ratio of enhanced regions compared to tumor background (N=3). **(E)** *(top)* Antares BLI in mice on day 11 following intravenous injection of Nalm6 cells, *(bottom)* FLuc BLI in mice 24 hrs post-intravenous injection with irradiated engineered NK-92 cells with quantification of signal in midsection (tumor-bearing: N=3; healthy: N=4). **(F)** FLuc BLI of excised organs 48 hrs post-injection of NK-92 cells. Data are presented as mean ± SD (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$).

Tracking CAR-NK and Cancer Cells in Mice using Dual Bioluminescence Imaging

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Introduction: Chimeric antigen receptor (CAR) immune cell therapy is being extensively explored for the treatment of many types of cancer. A CAR directs an immune cell to a particular cancer cell-surface antigen and upon binding instructs the immune cell to kill the cancer cell. Compared to the most studied CAR-T cells, CAR-natural killer (CAR-NK) cells offer some significant advantages. NK cells naturally kill cancer cells and not normal cells, initial evidence suggest their use is associated with less side effects, and they can be made from any donor providing the ability to make an off-the-shelf product. To more effectively evaluate new CAR-NK designs in preclinical models it would be beneficial to track both the CAR-NK cells to assess whole-body tumour-homing and persistence, as well as the cancer cells to assess treatment response. Bioluminescence imaging (BLI) is affordable and high throughput and has orthogonal luciferases available for tracking more than one cell population at a time. Our goal was to develop dual BLI tools to non-invasively track CAR-NK cells and our targeted cancer cells over time in the same mice.

Methods and Results: CD19-CAR-NK92 cells were designed to target and kill CD19+ Nalm6 leukemia cells. NK-92 cells were transduced using a lentivirus co-expressing an CD19-targeted CAR, GFP, Firefly luciferase (Fluc) for BLI, and OATP1B3 for future MRI. Following transduction, GFP+ CD19-CAR-NK92 cells were sorted by FACS to 100% purity. In vitro BLI confirmed functional expression of FLuc. Nalm6 cells were engineered to express the BLI reporter Antares using a separate lentivirus and also sorted to 100% purity. In vitro Nalm6 killing assay indicated after coculture with CD19-CAR-NK92 cells for 24 hours, Nalm6 cells survival rate was 36.6% of that with NK92 naïve coculture suggesting CD19-CAR was successfully engineered into NK92 cells.

Eight nod-scid-gamma (NSG) mice (6-week-old) were divided into 2 groups and all mice were injected with $2x10^6$ Nalm6-Antares cells via the tail-vein. Tumor growth was monitored by Antares BLI using the substrate FFZ. At 7, 12, and 17 days after cancer cell injection, mice were intravenously injected with $15x10^6$ irradiated CD19-CAR-NK92 cells or saline alone. The CD19-CAR-NK92 cells were monitored by FLuc BLI using the substrate D-luciferin. The Antares BLI result showed there was a significant decrease of tumor size after CARNK92 treatment on the right body part compared to control group ($p<0.05$), however, there was no significant different between the 2 groups on the whole-body comparison. FLuc BLI result clearly indicated all IV injections were successful and homing of CD19-CAR-NK92 cells was seen head region.

Discussion: Our work provides an efficient dual BLI system to non-invasively track both CAR-NK cells and targeted cancer cells. This imaging tool will provide important information on immune cell fate and treatment responses in preclinical models and should be broadly applicable for other CAR designs and other cancer models.

Poster #27

Chemical Programming of Macrophages for Targeted Tumour Immunotherapy

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A rising concept in the field of immunotherapy is the idea of genetically engineering immune cells to display engineered receptors that can target tumour antigens. These have shown great success in targeting tumour cells and decreasing tumour burden in mouse models and in *in vitro* studies. However, these types of therapies require that each patient's immune cells be engineered individually. This process is both work and resource intensive and therefore limits the availability of this treatment. My project works to overcome this issue by developing a method to program endogenous immune cells by treating patients with synthetic proximity inducing molecule instead of through individual genetic engineering.

My project focuses on the chemical programming of macrophages, a type of immune cell, isolated from cancer patients using a molecule called a chemical immune programmer (CIPs). By attaching a CIP with a tumour targeting molecule directly to an activating receptor on macrophages we can program immune cells through binding induced activation without the need for genetic engineering (Figure 1). Our CIPs contain a macrophage targeting domain to allow for binding induced activation of the macrophages. The CIP also includes a click handle, which can be used to attach any tumour targeting molecule of choice directly in vivo, such as already commercially available therapeutics to the surface of the macrophage. Once re-implanted into the patient, the tumour targeting molecule can promote macrophage tumor engagement and stimulate tumoricidal function. Flow cytometry experiments have shown that CIPS are able to specifically and effectively bind immune receptors on the surface of model macrophage cell lines. We were also able to use the click handle on the CIP to specifically label the macrophage cell surface with a targeting molecule. Further, CIPs were able to induce macrophage activation and promote antibody-dependent cellular phagocytosis.

These experiments have also shown that direct engagement of the receptor using our CIP is more effective than antibody-mediated engagement. This suggests that out platform maybe more effective than current antibody treatments. The click handle allows this system to be modular, resulting in a flexible novel immunotherapeutic treatment that can target many different tumour types. These results point to the development of a novel immunotherapeutic that is more versatile, accessible and effective.

Investigating the role and regulation of galectin-12 in a model of neutrophil-like differentiation of human acute promyelocytic leukemia HL-60 cells

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Introduction: Galectin-12 is a tissue-specific and lipid droplet-binding galectin with altered expression observed in several cancers. Previous studies found that lower mRNA expression of galectin-12 was linked to poorer prognosis in patients with acute myeloid leukemia. These observations suggest that galectin-12 may be classified as a tumor suppressor considering its involvement in the cell differentiation program of such cells as adipocytes and neutrophils. However, in the case of neutrophilic differentiation, the role of galectin-12 is still unclear due to a variety of phenotypes observed in circulation and tumor tissues. We hypothesized that galectin-12 can serve as a marker of different phenotypes of neutrophils and sought to compare the mechanisms of its regulation in human acute promyelocytic leukemia HL-60 cells undergoing neutrophil-like differentiation in response to all-trans retinoic acid (ATRA) and dimethyl sulfoxide (DMSO).

Methodology: RT-PCR and ELISA methods were used to analyze changes in the expression of galectin-12 at gene (*LGALS12*) and protein levels including the secretion of this galectin. Neutrophil-like differentiation of HL-60 cells was evaluated by examining nuclear morphology, expression of *NCF1*/*NCF2* genes, and functional responses of cells (H_2O_2 generation) under *N*-formyl-L-methionyl-leucyl-phenylalanine (fMLP) or phorbol 12-myristate 13-acetate (PMA) stimulation. Biochemical inhibitors were used to examine the roles of *O*-GlcNAc homeostasis and unconventional secretion mechanisms in galectin-12 regulation. Bioinformatics analysis using available RNA-Seq data was used to compare gene expression profiles between ATRA and DMSO-differentiated neutrophil-like HL-60 cells. BODIPY 493/503 and fluorescence microscopy were used to stain for lipid droplets in HL-60 cells.

Results: ATRA-induced neutrophil-like differentiation of HL-60 cells led to a significant increase in *LGALS12* expression, while DMSO induced a significant decrease. Intracellular concentrations of galectin-12 did not change in both cases as detected by ELISA, however, galectin-12 secretion was blocked in cells treated with either ATRA or DMSO for 72 h. Remarkably, both ATRA and DMSO induced an increase in lipid droplet accumulation inside HL-60 cells. No significant changes in galectin-12 secretion levels were observed for HL-60 cells treated with inhibitors of *O*-GlcNAc cycle enzymes or exosomal/microvesicular secretion but a modulator of secretory autophagy significantly increased galectin-12 flux out of cells. There were significant differences between ATRA- and DMSO-induced phenotypes of HL-60 cells in terms of low versus high magnitude of functional responses to fMLP and expression of formyl peptide receptor 1 (*FPR1*), respectively, while the response to PMA was consistent between these treatments. Gene set enrichment analysis found that ATRA and DMSO neutrophil-like cells significantly differed for hallmark terms covering E2F targets, G2M checkpoints, and MYC targets among others.

Conclusions: These findings provide evidence that ATRA and DMSO induce differentiation of HL-60 cells into different phenotypes of neutrophil-like cells with variable expression of galectin-12 as a specific biomarker and lipid droplet-binding molecule**.** The association of these phenotypes with clinically relevant aspects of leukemia cell biology is an emerging target for our upcoming research.

THANK YOU

Guest Speakers

Jeanette Boudreau, PhD
Jonathan Bramson, PhD
Marcus Butler, MD
Tracy McGaha, PhD
John Ronald, PhD
Ian Watson, PhD

Oral Presentation Judges

Jeanette Boudreau, PhD
Jonathan Bramson, PhD
Marcus Butler, MD

Hosts

Hasti Ghomali, MSc. Cand.
Amanda Liddy, MSc. Cand.
Saman Maleki, PhD (Conf. Chair)
John Ronald, PhD (Conf. Co-Chair)
Rafael Sanchez-Pupo, Post Doc

Administration

Priscilla De Luca
Margaret Dow
Lisa Michienzi
Kimberly Scott

Poster Presentation Judges

Jeanette Boudreau, PhD
Jonathan Bramson, PhD
Marcus Butler, MD
Mansour Haeryfar, PhD
Peeyush Lala, PhD
Ting-Yim Lee, PhD
John Lenehan, MD
Tracy McGaha, PhD
John Ronald, PhD
Trevor Shepherd, PhD
Parisa Shooshtari, PhD
Jonathan Thiessen, PhD
Ian Watson, PhD
Xiufen Zeng, PhD

...AND TO
THE
REGISTRANTS