McGill | Department of Pathology

Finlayson Research Day

‘Science in Pathology’

June 14th, 2024

Research Institute of the McGill University Health Centre (RI-MUHC) Glen Site Amphitheatre
1001 Décarie Blvd.

AND

McGill University Faculty Club
3450 McTavish Street

Finlayson Research Day

Finlayson Day is held each year by the Department of Pathology in memory of **Dr. Morrison H. Finlayson** who was on the staff at McGill for more than 20 years, both as a pathologist at the Montreal General Hospital and an Associate Professor in the Department of Pathology.

Dr. Finlayson received his MB, ChB from the University of Edinburgh and his residency training in neurology and pathology at the University of Alberta and at McGill. His major research interest was neuropathology, and he published numerous research articles and case reports in this field. He was also an active member of many scientific associations and became President of the Canadian Association of Neuropathologists and Vice-President of the International Society of Neuropathology. For many years, Dr. Finlayson was also the Director of the McGill Residency Training Program in Pathology, and it is in this role that he is remembered with particular affection. His concerns for the quality of education and for the well-being of each individual during the period of their training were deeply appreciated. For these reasons, the Department of Pathology holds an annual day in his honour.

Following a special lecture by an invited speaker, brief research presentations are given by members currently enrolled in Residency Training and in the Graduate Studies Program. A prize is awarded for the best short presentation in each category, but the main goal is to provide a relaxed forum for the exchange of ideas between members of the department who are investigating a wide range of different topics, and often work in separate geographical locations within the university hospital complex. This interaction is further encouraged by the subsequent social events held in a very congenial environment.

Past Guest Speakers

1985/86 Dr. Joseph GILBERT
1986/87 Dr. Malcolm SILVER
1987/88 Dr. John CRAIGHEAD
1988/89 Dr. David MURRAY
1989/90 Dr. Mary-Ellen KIRK
1990/91 Dr. Shao-Nan HUANG
1991/92 Dr. Jim HOGG
1992/93 Dr. Robert KISILEVSKY
1994/95 Dr. William THURLBECK
1995/96 Dr. Dante SCARPELLI
1996/97 Dr. Laurence E. BECKER
1997/98 Dr. David LOUIS
2005/06 Dr. Michael GIMBRONE
2006/07 Dr. Ming-Sound TSAO
2007/08 Dr. Marc LADANYI
2008/09 Dr. Nancy Lee HARRIS
2009/10 Dr. Stanley J. ROBBOY
2010/11 Dr. Louis DUBEAU
2011/12 Dr. Robert ODZE
2012/13 Dr. Jonathan EPSTEIN
2013/14 Dr. Stuart SCHNITT
2014/15 Dr. John HART
2015/16 Dr. Robert KURMAN
2016/17 Dr. William FAQUIN
2017/18 Dr. Thomas KRAUSZ
2018/19 Dr. Matt VAN DE RIJN
2019/20 Dr. Ralph HRUBAN *virtual*
2020/21 Dr. Rhonda K. YANTISS *virtual*
2021/22 Dr. Logan WALSH
2022/23 Dr. Blake GILKS

Guest of Honour 2024

Dr. Sylvia L. ASA, MD, PhD

Dr. Sylvia Asa obtained her MD from the University of Toronto in 1977 and her PhD in Pathology from the same institution in 1990 after completing a residency in Pathology in 1982. She joined the Faculty of Medicine at the University of Toronto in 1985 and was promoted to Professor in the Department of Laboratory Medicine and Pathobiology in 1998. She was appointed as the Pathologist-in-Chief at the University Health Network in Toronto from 2000 to 2015. In 2019 she joined the Faculty at Case Western Reserve University and University Hospitals Cleveland.

A Clinician-Scientist with a focus on Endocrine Pathology, her research aims to identify the basis for development of endocrine tumours, to improve diagnostic tests and to identify targets for therapy of those diseases. She has published more than 600 scientific articles, written 7 books, co-edited 9 books and written more than 150 book chapters. She serves on numerous editorial boards of scientific journals and has given over 300 invited lectures. She is an Expert Member of the WHO 5th Edition Classification of Tumours Editorial Board for Endocrine and Neuroendocrine Tumours.

Dr. Asa has served as President of the Endocrine Pathology Society (1997-98) and the US-Canadian Academy of Pathology (2005-06). She has received many awards and honors, including the Arthur Purdy Stout Society of Surgical Pathologists, the Novartis Canada Senior Scientist Award, the Professor C.F.A. Culling Memorial Lecture Award of the National Society for Histotechnology, the ICRF Woman of Action Award, the F.K. Mostofi Distinguished Service Award of the US-Canadian Academy of Pathology, honorary fellowship in the Royal College of Pathologists, a Lifetime Achievement award from the College of American Pathologists and a Distinguished Physician award from University Hospitals Cleveland.

As head of the largest academic pathology department in Canada from 2000 through 2015, Dr. Asa has made innovative changes to the practice of the discipline, with emphasis on subspecialisation, molecular diagnostics, biobanking, automation, pathology informatics and digital pathology. She has emphasized the importance of education and research. To ensure public knowledge of the role of Pathology and maintain a direct connection with patients, Dr. Asa is also a consultant to several patient groups.

Faculty Speaker 2024

Dr. Basile Tessier-Cloutier, MD

2024-2025 Award Recipient, FRQS Clinical Research Scholar

Dr. Basile Tessier-Cloutier completed his medical training at McGill University in Montréal and later moved to Vancouver for a combined residency in Anatomical Pathology and Molecular Oncology Research Fellowship at the University of British Columbia. He followed his pathology training by a Clinical Fellowship in Gynecologic Pathology at Memorial Sloan Kettering Cancer Center. Now an Attending Gynecologic Pathologist at the McGill University Health Centre (MUHC) and a Principal Investigator at the Research Institute (RI-MUHC), his research focuses on the morphologic and molecular profiling of undifferentiated gynecologic malignancies and experimental model development.

June 14th, 2024

Finlayson Research Day Program

Morning Session

MUHC-Glen Dept. of Pathology (Rm E04.4047)

9:00 – 11:15 AM Slide session/cases at multi-header [Dr. Asa and Residents]

11:30 – 12:45 PM Lunch [Dr. Asa and Invitees]

Afternoon Session

RI-MUHC Amphitheatre (Rm ES1.1129)

1:00 – 1:10 PM Opening remarks [Dr. Lili-Naz Hazrati, Chair]

1:10 – 2:00 PM Finlayson Memorial Lecture [Dr. Sylvia Asa]
Looking beyond the microscope to predict the future

2:00 – 2:30 PM Faculty Talk [Dr. Basile Tessier-Cloutier]
Understanding malignant proliferations with morphological dedifferentiation: a universal frustration!

-Moderator: Dr. Julia Burnier-

Mini-Symposium I: Residency Program Research

[C1] 2:30 – 2:45 PM [Dr. Mina Farag, PGY-4] *Characterization of HER2-altered colorectal carcinomas in North American patients: a case-control study of clinicopathologic and molecular features of HER2-amplified and HER2-mutated colorectal carcinomas compared to their HER2 wild-type counterparts* Abstract

[C2] 2:45 – 3:00 PM [Dr. Émilie Pichette, PGY-1] *Workflow for slide-block alignment and tissue microarray generation* Abstract

[C3] 3:00 – 3:15 PM [Dr. Saba Alsaddah, PGY-3] *Molecular landscape of colorectal cancer at a Quebec health care centre using next-generation sequencing for liquid biopsy-based targets* Abstract

[C4] 3:15 – 3:30 PM [Dr. André Lametti, PGY-4] *A clinicopathological and artificial intelligence-derived screening model as a tool for distinguishing patients with non-Hodgkin lymphomas from inflammatory hepatic diseases: a multicentric learning approach* Abstract

3:30 – 3:45 PM Coffee Break

Mini-Symposium II: Graduate Program Research

[R1] 3:45 – 4:00 PM [Tadhg Ferrier, PhD4] *HPV ctDNA as an effective tool for monitoring patients with cervical cancer and dysplasia* Abstract

[R2] 4:00 – 4:15 PM [Leonardo F. Jurado, PhD3] *A fungal-derived adjuvant amplifies the antitumoral potency of BCG* Abstract

[R3] 4:15 – 4:30 PM [Rewati Prakash, PhD3] *Oxaliplatin and 5-fluorouracil cause chemotherapy-induced senescence in low-grade serous ovarian cancer cells* Abstract

[R4] 4:30 – 4:45 PM [Zhaoping (Aaron) Ju, PhD2] *Role of Hippo signaling effectors Yap & Taz in gastric metaplasia and tumorigenesis* Abstract

[R5] 4:45 – 5:00 PM [Meagan Cobb, MSc1] *Development of multi-omic liquid biopsy assay for the pre-operative distinction of uterine leiomyomas and leiomyosarcomas* Abstract

[R6] 5:00 – 5:15 PM [Willem Rijnbout-St. James, PhD2] *The aryl hydrocarbon receptor: implications in pulmonary fibrosis* Abstract

5:15 – 5:20 PM Voting Period for Best Clinical Presentation and Best Research Presentation (QR code on the next page)

Evening Reception

McGill Faculty Club (3rd Floor, Billiards Room)

6:00 – 7:00 PM Cocktails and Hors d'oeuvres

7:00 – 10:00 PM Welcome, Dinner Service & Awards Ceremony

Welcome Remarks	Dr. Lili-Naz Hazrati, Chair
Staff Teacher of the Year Resident Teacher of the Year Resident of the Year Residents Class of 2024 Best Clinical Presentation at Finlayson Day	Presented by Dr. Baharak Khadang, RPD
Graduating MSc and PhD Students Best Research Presentation at Finlayson Day	Presented by Dr. Carolyn Baglole, GPD
Moy Fong Chen Travel Awards (2023)	Presented by Dr. Moy Fong Chen

Voting for Best Trainee Presentations

Responses are ANONYMOUS. Please cast one vote in each category: Best Clinical Presentation [C1-C4] and Best Research Presentation [R1-R6].

https://forms.office.com/r/UkQUNZGbee

Abstract Booklet

[C1] CHARACTERIZATION OF HER2-ALTERED COLORECTAL CARCINOMAS IN NORTH AMERICAN PATIENTS: A CASE-CONTROL STUDY OF CLINICOPATHOLOGIC AND MOLECULAR FEATURES OF HER2-AMPLIFIED AND HER2-MUTATED COLORECTAL CARCINOMAS COMPARED TO THEIR HER2 WILD-TYPE COUNTERPARTS

Mina Farag*

Background: While human epidermal growth factor receptor 2 (HER2, ERBB2) alterations have been long established as an oncogenic driver and therapeutic target in breast and gastric cancers, its role as a biomarker in colorectal cancer (CRC) has only recently been investigated. Essential heterogenicity exists among studies, compounded by use of variable testing methods and IHC scoring systems. Additionally, there is scarce information about the clinical parameters of HER2-altered CRC patients. Thus, we conducted a matched case–control study of molecularly identified HER2-altered CRC patients compared to HER2 wild-type controls from two large academic centers in North America.

Methods: Patients with CRC that underwent next-generation sequencing (NGS) analysis between 2013 and 2023 were selected, in each case with HER2 alteration matched by age and sex to one HER2 wild-type control and compared with respect to their clinical, pathologic, and molecular characteristics. Kaplan–Meier estimates of cumulative survival over time were obtained for both groups. Molecular HER2 status was correlated to IHC expression, comparing the currently available scoring systems (HERACLES, gastroesophageal and breast scores).

Results: Overall, 106 CRCs were selected. The 53 cases and 53 controls were the same in mean age 59.1 years and 60% Male. NGS identified 30/53 patients with HER2 mutation and 23/53 patients with HER2 amplification by copy number variation analysis. Among evaluated CRC risk factors, active smoking was enriched in HER2-altered cohort (<0.001). The tumor location was not significantly different between cases and controls (left side: 25 vs. 30, p=0.2). However, multifocal tumors were predominately identified among HER2-altered patients (8/9 multifocal cases, p=0.055). All HER2-amplified CRCs had intact MMR while HER2-mutated CRCs showed loss of PMS2 (3), MLH1/PMS2 (1) and MSH2/MSH6 (1), (p=0.04). Only 7 HER2-positive patients received anti-HER2 treatment. HER2-altered CRCs showed inferior overall survival compared to HER2-negative CRCs (mean [CI] 4.6 [3.7-5.6] vs. 8.6 [6.7-10.0] years, respectively; p=0.06). A subset of cases was additionally tested for HER2 expression by IHC. All HER2-amplified CRCs showed 3+ HER2 expression (p<0.001), while mutated and control cases were all negative (score 0). All scoring systems had perfect positive linear relationships (r=1; p<0.001).

Conclusion: This matched case-control multicenter study outlined multiple novel clinicopathologic and molecular features of HER2-altered CRCs. HER2 IHC predicted HER2 amplification, irrespective of scoring system used. HER2-mutated CRCs had distinct clinicopathologic profiles, suggesting a distinct entity.

*Mina Farag[1,2], Pari Jafari[3], André Lametti[1,2], Saba Alsaddah[1,2], Andrea Gomez[2], Victoria Marcus[1,2], Pierre-Olivier Fiset[1,2], Namrata Setia[3], Greta Evaristo[1,2]

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[C2] WORKFLOW FOR SLIDE-BLOCK ALIGNMENT AND TISSUE MICROARRAY GENERATION

Émilie Pichette*

Background: Traditionally, a tissue microarray (TMA) is prepared by circling an area of interest on a formalin-fixed paraffin-embedded (FFPE) slide, which is then transferred onto the corresponding block to mark the area to be cored. However, tissue processing including microtomy and section flotation can have an impact on the size and shape of the tissue on the slide, causing discordance with the block. Our aim was to design a workflow for TMA preparation to improve tumor sampling by considering the deformation that occurs during the process of slide generation from a block.

Methods: 129 cases of esophageal and gastric adenocarcinoma were selected, with a total of 238 blocks and corresponding FFPE hematoxylin and eosin-stained slides. The slides were scanned at 40X magnification using a digital slide scanner. The blocks were scanned using a flatbed scanner, and a corresponding TIFF file was created for each block image. Slides were first annotated on QuPath using a machine-learning algorithm, which automatically outlined the entire tissue. A staff pathologist reviewed the outline and added a 5 mm diameter circular annotation identifying an appropriate area of tumor to core. Using the Warpy extension and Fiji, an open-source image processing software, scanned slide images with annotations were overlapped to the block TIFF images, allowing for slide annotations to be aligned and transferred to the block. Mean, standard deviation and paired t-tests were performed for each variable, comparing the slide and block groups.

Results: The mean area expansion between slide and block was 1.3 for both circled tumor and total tissue (SD 0.16 and 0.12, respectively). Differences in area of both circled tumor and total tissue as well as mean diameter (mean of largest and smallest diameter) and circularity (calculated as 4π*area/perimeter^2) of the tumor annotation were statistically significant between the slide and block groups, with a p value of <0.0001. Annotations were printed on clear labels to identify areas for TMA preparation.

Conclusion: Overall, we show that in our sample of 129 cases there was a 30% increase in tissue surface area from block to slide, associated with significant deformation of the tissue. This highlights the pitfalls of the current method of TMA preparation, and the need for an additional alignment step in the process to improve tumor sampling.

Source of Funding: Canadian Cancer Society, Astellas.

*Émilie Pichette[1], Chandler Ochs[1], Nickolaos Sagos[1], Alfred Cuellar[1], Ajay Rajaram[1], Greta Evaristo[1], Chelsea Maedler-Kron[1], Victoria Marcus[1], Lorenzo Ferri[2], Pierre-Olivier Fiset[1]

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[C3] MOLECULAR LANDSCAPE OF COLORECTAL CANCER AT A QUEBEC HEALTH CARE CENTRE USING NEXT-GENERATION SEQUENCING FOR LIQUID BIOPSY-BASED TARGETS

Saba Alsaddah*

Background: Biomarker testing for colorectal cancer (CRC) is important for decision making of advanced disease. This study aims to describe the molecular epidemiology of CRC in patients who underwent next-generation sequencing (NGS) and develop a liquid biopsy-based biomarker utilizing circulating tumor DNA (ctDNA).

Design: Patients were tested reflexively on metastatic biopsies and resection specimens. NGS testing was performed with the Ampliseq for Illumina Focus Panel. Sequencing data and clinicopathological parameters were collected from January 2019 to June 2023.

Results: The cohort consisted of 1046 patients. The most common mutations identified were *KRAS* (41.9%), *BRAF* (12%), *APC* (5.3%), *NRAS* (4.3%), *PIK3CA* (4.2%) and *ERBB2* (1.3%). In 30.4%, no mutation was detected. *BRAF* was associated with older mean age of 74 (SD 11) years and females (58.7%). In contrast, *ERBB2* was more common in males (78.6%) and younger mean age of 59 (SD 14) years. *BRAF* was more common in right-sided disease ($p<0.001$). Metastatic specimens were associated with *KRAS* (45.7%). *BRAF* mutations were found in 66% of MLH1/PMS2 loss cases ($p<0.001$). Subset analysis revealed improved survivorship in patients with *BRAF* (41.5 [CI 32.0-50.9] months) compared to *APC* (33.1 [CI 23.1-43.2] months) and *KRAS* (34.6 [CI 30.1-39.1] months). Subsequently, probes and primers were successfully designed for ctDNA detections via digital droplet PCR for a subset of 43 patients.

Conclusion: Our study is concordant with larger CRC sequencing studies and highlights the potential of liquid biopsy-based biomarkers. The detection of targetable mutations via NGS or ctDNA offers potential in guiding personalized therapeutic decisions.

*S. Alsaddah[1], M. Farag[1], M. De Nobrega[2], K. Dickinson[2], V. Marcus[1], G. Evaristo[1], C. Maedler-Kron[1], S. Camilleri-Broët[1], A. Gomez[1], A. Spatz[1], J. Burnier[1,2], L. Lee[3], PO. Fiset[1]

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[C4] A CLINICOPATHOLOGICAL AND ARTIFICIAL INTELLIGENCE-DERIVED SCREENING MODEL AS A TOOL FOR DISTINGUISHING PATIENTS WITH NON-HODGKIN LYMPHOMAS FROM INFLAMMATORY HEPATIC DISEASES: A MULTICENTRIC LEARNING APPROACH

André Lametti*

Background: Primary hepatic non-Hodgkin lymphomas (NHL) are rare, with nonspecific and often subtle clinical presentations. Differentiating NHL from an inflammatory process represents a diagnostic challenge, requiring an extensive workup, which can delay management and lead to rapid clinical deterioration. A reliable method for triaging cases with a higher likelihood of NHL is critical for rapid evaluation. We investigated a multimodal approach using several clinicopathological parameters and artificial intelligence (AI) as an innovative avenue for expedited identification of hepatic NHL.

Methods: Patients with NHL and other conditions inducing prominent inflammatory responses from four North American academic centers were reviewed for different clinical parameters and liver biopsy findings. For AI-based analysis, whole slide images (WSI) of available H&E-stained slides were randomly assigned to training (70%) and validation (30%) sets. A weakly supervised learning paradigm derived from attention-based multiple-instance learning was applied to the training set. The performance of the trained model was tested on the validation group.

Results: Between 2016 and 2023, 146 patients were included. The mean (SD) age was 59.9 (16.8) years, and 53% were male. NHL cases comprised 53% diffuse large B-cell, 11% T-cell, 9% marginal, 8% follicular, and 19% other lymphomas. Non-NHL controls, used as negative ground truth in the AI training, comprised of 60% inflammatory liver diseases, 15% non-NHL tumors, 15% normal biopsies, and 10% other diagnoses. In clinically unsuspected cases, NHL had significantly worse survival compared with non-NHL (mean [CI] 22 [17-28] vs. 40 [29-51] months; $p=0.02$). ALT and ALP (xULN) were significantly lower in NHL compared with non-lymphoma (mean [SD] 1.9 [4.0] vs 6.6 [6.2]; $p<0.001$ and 1.5 [1.4] vs 2.2 [1.7]; $p=0.03$). ALT <x3 ULN showed 93% specificity and 75% NPV in NHL patients. The area under the ROC curve for discriminating NHL by ALT was 0.803 (95% CI: 0.7-0.9; $p<0.001$). The AI-trained model investigating liver biopsies without hyperparameter optimization was 73% accurate in classifying patients in the validation set, with the area under the ROC curve of 0.851.

Conclusion: This multicenter study outlined clinicopathologic features of hepatic NHL that can potentially be used along with low/no-annotation AI models as an efficient and reliable triaging tool to prioritize hepatic NHL diagnosis. Further integration of biochemical parameters will allow for model optimization.

Mina S. Farag[1,2], *André Lametti[1,2], Ajay Rajaram[1,2], Nadine E. Demko[3], Pari Jafari[4], Ruoji Zhou[5], Alison Xiaotang Du[5], Pierre-Olivier Fiset[1,2], Amine Benmassaoud[6], Farhad Maleki[7], Victoria Marcus[1,2], René P. Michel[1,2], Amy Chadburn[3], John A. Hart[4], Greta Evaristo[1,2]

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*Presenter

[R1] HPV ctDNA AS AN EFFECTIVE TOOL FOR MONITORING PATIENTS WITH CERVICAL CANCER AND DYSPLASIA

Sarah Tadhg Ferrier*

Background: Cervical cancer (CC) is the 4th most diagnosed cancer among women, with ~528,000 deaths annually. Despite effective methods for early detection of disease, current screening approaches have limitations: Pap tests require dedicated cytopathology infrastructure while Human Papillomavirus (HPV)-DNA testing lacks specificity for transient infections. Liquid biopsy has emerged as a minimally invasive method for detecting and monitoring various cancers. Despite advances in liquid biopsy techniques in cancer detection, little is known about the presence of circulating tumour (ct)DNA in different liquid biopsy samples in patients with CC and cervical dysplasia (CD). The goal of the study was to determine whether ctDNA identified from different liquid biopsies could be clinically useful in differentiating the spectrum of cervical lesions.

Methods: Blood, urine, and vaginal swabs were collected from 114 patients – 45 CC and 69 CD – at the McGill University Health Centre. Cell-free DNA was isolated and tested for HPV-ctDNA using ddPCR for HPV16/18/31/33/35/45/52/58. ctDNA results were compared to Pap HPV-DNA and clinical data. Longitudinal samples were obtained for 30 patients.

Results: HPV-ctDNA was detectable in patients with CC and CD in all liquid biopsy sample types tested. HPV-ctDNA was significantly more detectable in CC patients than CD patients. ctDNA detection correlated with extent of disease. Self-collection methods (urine and vaginal swab) were highly concordant with pap samples (κ=0.651,0.836). Plasma ctDNA concentration was found to be highly associated with disease stage ($P<0.005$). Patients showed significant reductions in HPV-ctDNA following treatment.

Conclusion: HPV-ctDNA is detectable in patients with CC and CD, with a significantly higher detection rate in CC. In plasma and urine, higher disease stage correlates with increased agreement between all sample types. Concordance between self- and physician-collected methods provides evidence for improved access to testing using self-collection methods. Treatment leads to reductions in ctDNA levels across all samples, pointing to utility of HPV-ctDNA as a tool for differentiating between CC and CD and monitoring treatment response.

*Sarah Tadhg Ferrier[1,2], Erica Mandato[1,2], Simane Warsame[2], Alexandra Bartolomucci[1,2], Hila Tabrizian[2], Fady Williamson Mansour[3], Basile Tessier-Cloutier[1,2], Xing Zeng[3], Kuang-Bo Joung[2], Shuk On Annie Leung[1,2,3], Julia Valdemarin Burnier[1,2,4]

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[R2] A FUNGAL-DERIVED ADJUVANT AMPLIFIES THE ANTITUMORAL POTENCY OF BCG

Leonardo F. Jurado*

Background: Bladder cancer (BC) is the ninth most common malignancy worldwide and the fifth most common in Europe and the USA. Bacillus Calmette-Guérin (BCG) intravesical immunotherapy is the established treatment. Yet, its mechanism of action remains poorly understood. Although BCG induces a complete response in a significant proportion of patients with high-risk tumors, 40 to 60% of patients experience recurrence within five years. Recently, we have demonstrated that systemic BCG vaccination enhances the innate immune response and host protection against both homologous (*M. tuberculosis*) (Kaufmann, *Cell* 2018) and heterologous (Influenza virus) infections (Tran, *Nat Immunol* 2024). Importantly, BCG protection was mediated via epigenetic reprogramming of hematopoietic stem cells (HSC) in the bone marrow (BM), which was transmitted to progenitor and innate immune cells inducing central trained immunity. Similarly, we have recently shown that repeated installations of BCG in patients with bladder cancer and a murine model resulted in long-term reprogramming of HSCs and progenitor cells (HSPCs) and the generation of effective trained immunity against cancer (Daman, *BioRxiv* 2024). This phenomenon has been termed Trained Immunity (TI). An example of TI in this context can be observed with the involvement of β-glucan (a fungal cell wall component), which reprograms HSCs and generates trained monocytes that promote host resistance against *M. tuberculosis* infection, enhancing host survival. Similarly, β-glucan has been shown to protect against various acute bacterial infections, fungi, and cancer. **Therefore, we hypothesize that the addition of β-glucan to the current BCG therapy will increase its antitumoral capacity via HSC-mediated Trained Immunity.**

Methods: To test this hypothesis, we investigated the impact of intravesical administration of BCG and/or β-glucan on HSPCs in the BM and the subsequent generation of innate immune cells with anti-tumoral activity in an orthotopic bladder cancer model.

Results: Unexpectedly we found that while the treatment with BCG or β-glucan resulted in ~50% increase in survival, the combination of both BCG/β-glucan increased survival to 100% with no detection of tumors in the bladder upon follow-up. Multiomic single nucleus analysis revealed that BCG or β-glucan reprogramed hematopoiesis, with skewing of HSPCs towards granulopoiesis and the generation of trained neutrophils. However, the magnitude and maintenance of trained neutrophils was significantly augmented in the dual therapy with BCG/β-glucan and featured a distinguishing molecular program. Analysis of neutrophil ontogeny in the bladder revealed that the treatment with BCG/β-glucan epigenetically and transcriptionally imprinted neutrophils in the BM with a predominant immature phenotype that, following their infiltration into the bladder, were able to resist converting to pro-tumor neutrophils (Ng, *Science* 2024).

Conclusion: Our findings reveal that the incorporation of β-glucan alongside the current BCG treatment for bladder cancer significantly enhance the efficacy of BCG while reducing the number of installations required. Given that β-glucan has been used in different human clinical trials, its safe usage in conjunction with BCG for treating patients with bladder cancer is highly feasible. Whether this combination bacterial/adjuvant therapy is suitable for other solid tumors requires further investigation.

*Leonardo F. Jurado[1], Vanessa M.S. Ross[1], Kristina Nikolaou[1], Kim A. Tran[1], Erwan Pernet[1], Renaud Prevel[1], Nargis Khan[1], Andrew W. Daman[2], Jin-Gyu Cheong[2], Oleg Loutochin[3], Victor A. McPherson[3], Eva Kaufmann[1], Michael S. Glickman[4], Steven Z. Josefowicz[2], Maziar Divangahi[1]

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[R3] OXALIPLATIN AND 5-FLUOROURACIL CAUSE CHEMOTHERAPY-INDUCED SENESCENCE IN LOW-GRADE SEROUS OVARIAN CANCER CELLS

Rewati Prakash*

Background: One of the greatest setbacks in treating low-grade serous ovarian cancer (LGSOC) is the poor response to standard chemotherapy agents. Oxaliplatin (OXP) has been used as an off-label treatment for ovarian cancer and has significantly more tolerable side effects compared to the standard chemotherapy regimen. OXP is generally provided in conjunction with 5-Fluorouracil (5-FU) as both drugs are known to work synergistically together in colorectal cancer. Prior studies have shown that OXP and 5-FU can independently cause chemotherapy-induced senescence (CIS) in multiple cancer types. Here we investigate whether CIS occurs in LGSOC when treated with OXP/5-FU.

Methods: Experiments were completed using three LGSOC cell lines: VOA6406, VOA7681 and VOA1056. Short-term viability, long-term clonogenic growth and recovery capabilities of the cells following treatment with OXP and 5-FU were assessed. Development of CIS was measured through multiple primary senescence hallmarks. Elevated activity of senescence-associated β-galactosidase (SA-β-Gal) and altered morphology was measured by X-gal staining and light microscopy. Further cytoplasmic structural changes were measured through caveolin-1 immunostaining and western blotting. Ki67 immunostaining was used as a marker of proliferation. Protein expression changes associated to CIS were evaluated by western blotting. Induction of oxidative stress by OXP and 5-FU was assessed using a fluorescence-based oxidative stress assay.

Results: Co-treatment of the LGSOC cells with OXP and 5-FU resulted in significantly reduced cell proliferation as well as poor clonogenic capacity; however, there was no reduction in viability. Interestingly, OXP/5-FU treated VOA6406 cells did not recover their growth across a period of 10 days whereas VOA7681 cells initially grew before plateauing at Day 4. SA-β-Gal and caveolin-1 expression was elevated in the cells upon treatment with both drugs and a visibly flatter and larger morphology developed. Ki67 staining was significantly reduced in cells treated with OXP and 5-FU when compared to the vehicle. Common senescence pathway protein expression was altered upon treatment including, p53, p21, pRb, p16, and p38MAPK. Additionally, OXP and 5-FU were able to induce high levels of oxidative stress in VOA6406 cells and to a slightly lesser extent in VOA7681.

Conclusion: In this study, the treatment of LGSOC cells with OXP and 5-FU reduced their proliferative capacity in both short and long-term settings. This reduction was accompanied by alterations to major markers of senescence. In addition, the combination of the drugs was able to induce the oxidative stress pathway without cell death, suggesting that this may be the driver of CIS in the OXP/5-FU treated LGSOC cells.

Source of Funding: Funded by the OvCAN (Ovarian Cancer Canada) program.

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[R4] ROLE OF HIPPO SIGNALING EFFECTORS YAP & TAZ IN GASTRIC METAPLASIA AND TUMORIGENESIS

Zhaoping (Aaron) Ju*

Background: Gastric cancer represents the third leading cause of cancer-related deaths globally, necessitating a deeper comprehension of its pathogenesis. Chronic infection with *Helicobacter pylori*, a bacterium that triggers inflammation and leads to lesions in the gastric epithelium, is a primary cause of gastric cancer. This process, known as oxyntic atrophy, is compensated by a hyperproliferative response as well as induction of metaplastic cell lineages, known as spasmolytic polypeptide-expressing metaplasia (SPEM). Although the link between SPEM and gastric cancer is well accepted, the mechanisms underlying metaplasia and its roles in gastric epithelium regeneration remain poorly understood.

Aims: This study aimed to investigate the role of Hippo transcriptional effectors, Yap and Taz, in driving gastric metaplasia. We hypothesized that Yap and Taz are crucial in driving gastric metaplasia and epithelium regeneration.

Methods: Yapfl/fl; Tazfl/fl; Clusterin-CreERT2 ($Clu^{Yap/Taz}$) mice underwent high dose tamoxifen (HDT) treatment, leading to Yap/Taz deletion and transient SPEM induction in the gastric epithelium. Yapfl/fl; Tazfl/fl; Atp4b-Cre ($Atp4b^{Yap/Taz}$) mice were also treated with HDT to induce SPEM. Phenotypic consequences of Yap/Taz depletion were assessed with immunofluorescence and immunochemistry following HDT treatment. Furthermore, gastric epithelial organoids were extracted from *Clu-CreERT* mice to assess the cell-autonomous functions of Yap/Taz. Finally, human gastric cancer tissue samples were analyzed for Yap expression.

Results: In the $Clu^{Yap/Taz}$ model, at Day 5 post-HDT (initiation phase of SPEM), both control and mutant mice exhibited increased CD44v9 (SPEM marker) expression and decreased H^+/K^+ ATPase (parietal cell marker) and Mist1 (chief cell marker). However, at Day 15 post-HDT (resolution phase), Yap/Taz mutants showed abnormally high CD44v9 levels, while H^+/K^+ ATPase and Mist1 remained low. Also, mutant mice displayed abnormal parietal cell morphology at Day 15 post-HDT. Furthermore, no viable gastric organoids lacking Yap/Taz were observed. Additionally, in $Atp4b^{Yap/Taz}$ mice, no abnormality in the cell markers mentioned above were observed. In human samples, Yap was highly expressed in SPEM and dysplasia regions, while much lower in areas of intestinal metaplasia (IM).

Conclusion: Our data suggested that Yap and Taz are involved in the late recovery stage of SPEM, rather than initiation. Also, Yap/Taz are essential for the survival of gastric epithelial organoids. Furthermore, Yap/Taz are dispensable in parietal cells during homeostasis and SPEM. Finally, an analysis of clinical samples suggested that tumorigenesis may depend on transient Yap and Taz suppression and/or may support a model whereby dysplastic cells originate directly from SPEM, instead of transitioning to IM.

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[R5] DEVELOPMENT OF MULTI-OMIC LIQUID BIOPSY ASSAY FOR THE PRE-OPERATIVE DISTINCTION OF UTERINE LEIOMYOMAS AND LEIOMYOSARCOMAS

Meagan Cobb*

Background: Leiomyomas (LM), frequently referred to as fibroids, are common benign tumours of the smooth muscle of the uterus which cause a variety of symptoms, including pain and pressure in the pelvis as well as disturbances in menstrual bleeding. Using clinical indications alone, it is challenging to accurately diagnose LM as they share clinical presentation with a rare, aggressive malignant tumour known as leiomyosarcoma (LMS). LMS is the most commonly diagnosed sarcoma with a disease-specific survival of around 50%. Pre-operative diagnosis of these tumors is also difficult because uterine lesions are rarely biopsied before surgery. To overcome these limitations, we hypothesize that a non-invasive circulating tumor DNA (ctDNA) test based on unique LMS- and LM-specific molecular markers will allow for an accurate pre-operative distinction between these tumours and will guide the choice of treatment.

Methods: We previously performed deep targeted sequencing and shallow whole genome sequencing of ctDNA extracted from plasma specimens of 7 LMS patients and 12 LM patients (PMIDs: 29463554, 32232185). To expand on the previous analyses, here we performed a new analysis of the first 4 nucleotide sequence (4-mer motifs) on the 5' end of the cell-free DNA fragments using the shallow whole genome sequencing data. The frequency of the possible 256 (4^4) 4-mer motifs was calculated using R programming language and normalized to the total number of reads in each specimen. Two-class differential analysis was applied to identify motifs enriched in LM and LMS (FDR < 0.05).

Results: Our previous studies demonstrated for the first time the feasibility of ctDNA detection in patients with LMS and LM. In LMS patients, ctDNA was detected in 6 of 7 patients with >98% specificity. In LM patients, ctDNA was detected in 6 of 12 patients with 76% specificity. To further increase the sensitivity of ctDNA detection in LMS and LM patients, we sought to incorporate fragmentomics as another type of ctDNA marker. Here, our new fragmentomic analysis identified 66 motifs significantly enriched and 21 motifs significantly decreased in cell-free DNA from LMS patients compared to LM patients. Further, motifs typically associated with DNASE1L3 nuclease activity (e.g., CCCA) were significantly decreased in LM patients compared to LMS patients.

Conclusion: We identified tumour type-specific markers in ctDNA of patients with LMS and LM. Next, we seek to incorporate DNA methylation markers to further increase the sensitivity of ctDNA detection in LMS and LM patients. We envision that combined detection of point mutations, CNAs, fragmentomic patterns and DNA methylation markers will allow us to develop a novel integrative multi-omic ctDNA assay that will allow for a highly accurate distinction between uterine LM and LMS based on a blood test.

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[R6] THE ARYL HYDROCARBON RECEPTOR: IMPLICATIONS IN PULMONARY FIBROSIS

Willem Rijnbout-St. James*

Background: Interstitial lung diseases (ILD) are a family of lung pathologies characterized by diffuse inflammation and fibrosis. The most common ILD is idiopathic pulmonary fibrosis (IPF), a fibrotic lung disease of unknown cause and for which patients have a median survival of 3 years from diagnosis. There is no cure for IPF and few treatment options, highlighting the need to better understand the molecular mechanisms driving the fibrotic response. Dysregulation of signaling pathways implicated in ECM production contribute to the persistent activation of lung fibroblasts, key effector cells in fibrosis, leading to overproduction of ECM proteins such as collagen that stiffen the lungs and impair gas exchange. One factor which may be involved in the development of lung fibrosis is the aryl hydrocarbon receptor (AhR), a transcription factor known to increase the expression of genes typically involved in drug metabolism. My preliminary data suggest that bleomycin-induced pulmonary fibrosis is dependent on the AhR and that the expression of COL1A1, an abundant collagen isotype overexpressed in fibrosis, is controlled by the AhR in lung fibroblasts. I hypothesize that the AhR is a novel regulator of profibrotic signaling. The purpose of this research is to further elucidate the involvement of AhR in lung fibrosis.

Methods: To assess how AhR may drive fibrosis *in vitro* and *in vivo*, mice/cells from AhR heterozygous and AhR knockout (KO) C57bl/6 mice were used. Primary mouse lung fibroblasts (MLFs) from AhR heterozygous and AhR KO mice were treated with TGF-β (5 ng/mL) or serum free media for 48 hours. COL1A1 and α-smooth muscle actin (α-SMA) protein was measured via western blot. Additionally, the synthetic AhR competitive antagonist, CH223191 (10 μM), was used to prophylactically inhibit the AhR in MLFs prior to TGF-β treatment. COL1A1 and α-SMA protein was again evaluated via western blot. To evaluate AhR dependent fibrotic outcomes *in vivo*, 8- to 12-week-old AhR heterozygous and KO C57bl/6 mice were treated with bleomycin (2 mg/kg in 50 μl PBS) or PBS (50 μl) via oropharyngeal aspiration. After 21 days, lungs were harvested from the mice and fixed in 4% paraformaldehyde for histological evaluation of fibrosis (Masson's Trichrome and H&E). Additionally, as confirmation of pulmonary exposure via intranasal administration for future experiments involving *in vivo* AhR antagonism, the endogenous AhR agonist 6-Formylindolo(3,2-b)carbazole (FICZ) (1 μg) was administered to mice intranasally and pulmonary CYP1A1 and COL1A1 expression was evaluated via qPCR.

Results: In response to TGF-β, AhR KO MLFs produced significantly reduced COL1A1 protein and significantly increased α-SMA compared to heterozygous MLFs ($p < 0.005$). Additionally, my preliminary findings indicate that bleomycin-induced pulmonary fibrosis develops in an AhR dependent manner. Furthermore, prophylactic CH233191-mediated AhR antagonism reduced TGF-β-driven COL1A1 protein production in AhR heterozygous MLFs ($p < 0.05$). Finally, intranasal administration of FICZ resulted in an observable increase in CYP1A1 and COL1A1 mRNA in the murine lung.

Conclusion: My preliminary findings suggest a pro-fibrotic role of the AhR, wherein AhR activity appears essential for fibroblast collagen synthesis and is required for bleomycin-induced pulmonary fibrosis. This work will identify novel, AhR-mediated mechanisms contributing to the development of fibrosis and could reveal novel biomarkers for monitoring disease progression as well as therapeutic targets. Moreover, the conservation of fibrotic mechanisms across different organs underscores the broader significance of this work. Insights gained from studying pulmonary fibrosis and AhR involvement may not only advance treatments for pulmonary fibrosis but also inform the development of therapies for other fibrotic diseases such as liver cirrhosis, systemic sclerosis, and cystic fibrosis. Considering the limited efficacy of existing therapeutic approaches for fibrotic disorders, this investigation offers promise for significantly enhancing fibrosis management strategies and enhancing health outcomes.

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