DEPARTMENT OF PATHOLOGY & LABORATORY MEDICINE

FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

ALBERTA PRECISION LABORATORIES
Leaders in Laboratory Medicine

THE UNIVERSITY OF CALGARY & ALBERTA PRECISION LABORATORIES

DEPARTMENT OF PATHOLOGY AND LAB MEDICINE

Present

RESEARCH DAY

Friday, June 21, 2024

Platform Presentations HSC Building – G500 (& Zoom)
Awards Reception – HRIC Atrium, Foothills Campus @6:15pm

UNIVERSITY OF CALGARY
CUMMING SCHOOL OF MEDICINE

DEPARTMENT OF PATHOLOGY & LABORATORY MEDICINE

ALBERTA PRECISION LABORATORIES
Leaders in Laboratory Medicine

THE UNIVERSITY OF CALGARY & ALBERTA PRECISION LABORATORIES, DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE'S

RESEARCH DAY

Friday, June 21, 2024

University of Calgary, Foothills Campus, Health Sciences Centre

8:15	**BREAKFAST (Hippocrates Atrium)**	
8:30 – 9:00	**POSTER SET UP (during breakfast)** **Hippocrates Atrium**	
9:30 – 9:40	Li, Roy (Qiaowang)	**DECODING NUCLEOTIDE SEQUENCE WITH HUMAN LINGUISTICS TO ADVANCE MUTATION ANALYSIS** Supervisor: Reda Alhajj
9:40 – 9:50	Li, Roy (Qiaowang)	**BIOLAKE: AN RNA EXPRESSION ANALYSIS FRAMEWORK FOR PROSTATE CANCER BIOMARKER POWERED BY DATA LAKEHOUSE** Supervisor: Reda Alhajj
9:50 – 10:00	Brett, Rhiannon	**THE EYES HAVE IT: FACTORS INFLUENCING TEMPORAL ARTERY BIOPSY SENSITIVITY** Supervisor: Dr Martin Hyrcza
10:00 – 10:10	Caires, Diogo	**MOLECULAR PROFILE OF ATYPICAL LEYDIG CELL TUMORS** Supervisor: Dr Tarek Bismar
10:10 – 10:20	Li, Hao	**SHORT-TERM FORMALIN FIXATION IMPAIRS ANTIGENICITY IN HUMAN LYMPH NODES FOR FLOW CYTOMETRY** Supervisor: Ryan Healey
10:20 – 10:30	Hu, Qian (Nancy)	**NON-HUMAN LEUKOCYTE ANTIGEN AUTOANTIBODIES ASSOCIATED WITH BIOPSY-PROVEN REJECTION AFTER KIDNEY TRANSPLANTATION** Supervisor: Dr Noureddine Berka
10:30 – 10:40	Seyedi, Sima	**ESTABLIISHING A NEW PROGNOSTIC PREDICTION MODULE FOR PROSTATE CANCER PATIENTS UNDER ACTIVE SURVEILLANCE PROGRAM USING A COMBINATION OF GENOMIC, HISTOLOGICAL AND DEEP LEARNING MODELS** Supervisor: Dr Tarek Bismar
10:40 – 10:50	Seyedi, Sima	**INCIDENCE OF MUTATIONS IN PROSTATE CANCER BY DNA SEQUENCING IN RELATION TO HISTOPATHOLOGICAL FEATURES** Supervisor: Dr Tarek Bismar
10:50 – 11:00	Farrington, Kyo	**EXPLORING THE LEARNING EXPERIENCES OF EARLY PATHOLOGY RESIDENCY TRAINING THROUGH THE LENS OF REALIST INQUIRY** Supervisor: Dr Amy Bromley
11:00 – 11:10	Rehman, Asia	**VALIDATION OF PROGNOSTIC STRATIFICATION BY NOVEL RISK GROUPS FOR UTERINE LEIOMYOSARCOMA** Supervisor: Dr Martin Koebel
11:10 – 11:20	Twa, David	**A CENTRALIZED, PROVINCIAL APPROACH TO GENE FUSION DETECTION** Supervisor: Erik Nohr
11:20 – 11:30	Twa, David	**FUSION PARTNER AGNOSTIC APPROACHES IMPROVE DETECTION OF TARGETABLE GENE FUSIONS IN THYROID CANCERS** Supervisor: Erik Nohr
11:30 – 11:40	House, Nicole	**CREATION OF A QUALITY ASSESSMENT FRAMEWORK: A CASE STUDY OF THE CALGARY AUTOPSY SERVICE** Supervisor: Dr Amy Bromley
11:40 – 11:50	Cai, Fangze	**COMMON PARKINSON'S DISEASE THERAPY LEADS TO FALSELY LOW URINE AND PLASMA CREATININE**

		Supervisor: Dr Michael Reid
11:50 – 12:00	Cai, Fangze	**THE EFFECT OF HEMOLYSIS ON POTASSIUM MEASUREMENT IN A COMMUNITY SETTING: THE MARCHING ERROR** Supervisor: Dr Jessica Gifford
12:00 – 12:10	Cai, Fangze	**REDUCING INAPPROPRIATE FECAL IMMUNOCHEMICAL TESTS** Supervisor: Dr Hossein Sadrzadeh
12:10 – 12:20	Salut, Norel	**EVALUATION OF MICROTOMY SECTIONING PROTOCOL FOR BREAST NEEDLE BIOPSIES IN ALBERTA PRECISION LABORATORY USING UNSTAINED SLIDES** Supervisor: Dr Hua Yang
12:20 – 12:30	Da-anoy, Annalyn	**A CASE REPORT OF TESTICULAR MAMMARY TYPE MYOFIBROBLASTOMA** Supervisor: Dr Tarek Bismar
12:30 – 1:15	**LUNCH (Hippocrates Atrium, Seating in G500)**	
1:15 – 1:30	**Introduction & Welcome:** Dr. Dylan Pillai **Moderator:** Dr. Martin Hyrcza **Adjudicators:** Dr. John Goldblum, Dr. Margaret Kelly, and Dr. Lawrence de Koning *Zoom link for offsite participation for platforms presentations:* https://ucalgary.zoom.us/j/96460151796?pwd=cEgZnHeLh9nHT30JN3bM7E9QiwVsXU.1	
1:30 – 1:45	House, Nicole	**AUTOPSY EDUCATION: REVIEW OF A LARGE CANADIAN ACADEMIC CENTRE** Supervisor: Dr Amy Bromley
1:45 – 2:00	Ezra, Sally	**EVIDENCE-BASED TEST UTILIZATION:TEST ORDERING PATTERNS IN FUNCTIONALMEDICINE AND COMMUNITY CLINICS, AND IMPLICATION SFOR LABORATORY STEWARDSHIP** Supervisor: Dr Hossein Sadrzadeh
2:00 – 2:15	Kalra, Amit	**EARLY DETECTION OF ACUTE GRAFT VERSUS HOST DISEASE BY ASSESSMENT OF THE IMMUITY RELATED TRANSCRIPTOME** Supervisor: Dr Faisal Khan
2:15 – 2:30	Kalra, Amit	**DIFFERENTIAL T CELL RECONSTITUTION AT THREE MONTHS POST HCT INFLUENCES THE IDENTIFICATION OF A LACK OF GVL REACTION** Supervisor: Dr Faisal Khan
2:30 – 2:45	McCoy, Christopher	**IDENTIFICATION OF AMYLOIDOGENIC PROTEINS IN FFPE TISSUES USING TARGETED LC-MS/MS** Supervisor: Dr Dennis Orton
2:45 – 3:00	Reich, Caitlan	**NASOPHARYNGEAL TUBARIAL GLANDS ARE MINOR SALIVARY GLANDS** Supervisor: Dr Martin Hyrcza
3:00 – 3:15	**BREAK (Hippocrates Atrium, Seating in G500)**	
3:15 – 3:30	Caires, Diogo	**EXTRAGLOMERULAR VASCULITIS AS AN INDEPENDENT PROGNOSTIC FACTOR IN ANCA-RELATED GLOMERULONEPHRITIS: A VALIDATION STUDY IN A CALGARY COHORT** Supervisor: Dr Hallgrimur Benediktsson
3:30 – 3:45	Robertson, Anna	**A NOVEL SIMULATION METHOD FOR TEACHING GROSS DISSECTION SKILLS IN ANATOMICAL PATHOLOGY** Supervisor: Dr Konstantin Koro
3:45 – 4:00	Gamallat, Yaser	**LAMTOR4 IS ASSOCIATED WITH LETHAL PROSTATE CANCER AND ITS KNOCKDOWN DECREASES CELL PROLIFERATION, INVASION, AND MIRGRATION IN VITRO** Supervisor: Dr Tarek Bismar
4:00 – 4:15	Twa, David	**PREVALENCE, TREATMENT, AND OUTCOME OF REAL-WORLD FUSION-POSITIVE NON-SMALL CELL LUNG CANCER (NSCLC) IN ALBERTA** Supervisor: Dr Erik Nohr

4:15 – 4:30	Brett, Rhiannon	**WHERE IN THE WORLD IS GNAQ MUTATED MELANOMA: A QUALITY ASSURANCE PROJECT** Supervisor: Dr Erik Nohr
4:30 – 5:30	G500	**Final Judging and Prize Allocation**
4:45 – 5:00	**Hippocrates Atrium Clean Up**	
5:00 – 6:00	HRIC Atrium	**Awards Cocktail Reception Set Up**
6:15	HRIC Atrium	**Hors d'oeuvres and Cocktails**
7:00	HRIC Atrium	**Awards Presentation**
8:30	**Reception Clean Up**	

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Category: _____Nucleotides modeling_____

DECODING NUCLEOTIDE SEQUENCE WITH HUMAN LINGUISTICS TO ADVANCE MUTATION ANALYSIS

Qiaowang Li[1], Yaser Gamallat [2,3,4], Reda Alhajj [1,5,6], Jon George Rokne[1] and Tarek A. Bismar[2,3,4,7]

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Introduction:

Applying nucleotide sequence analysis for clinical decision is a trending topic in current medical/bioinformatic research. For example, researchers tend to predict the degree of deterioration of prostate cancer by analyzing the DNA sequence and mutation biomarker. However, existing works are not yet satisfactory; some approaches involve manually selecting and observing mutation biomarkers, resulting in a lack of flexibility; some approaches simply apply a complex neural network for clinical decision-making, which leads to interpretability issues. This study presents a nucleotide sequence modeling method that interprets DNA sequence using human linguistics. The optimal goal of this study is to construct a robust numeric representation of nucleotide sequence for all mutation-related downstream tasking such as Gleason score prediction or case/control classification.

Methods:

This study applies three set of datasets for generating the sequence representation. Starting with a single layer transformer block. We first feed all DNA sequence from 23 human chromosomes to pre-train the transformer attention matrix. Once the pre-train is done, we apply public mutation data to fine-tune our tiny pre-trained transformer with knowledge distillation support from existing LLM such as DNABERT-2. The fine-tuning stages are also enhanced by greedy-epsilon algorithm to prevent the model from overfitting. We also pre-train 24 dynamic nucleotide networks, one for each chromosome, to accept nucleotide sequence with any lengths. As the final stage, we will apply a small set of private AS170 mutation data to validate the model.

Results:

The model returns a numeric representation [in vector format] given any nucleotide sequences where researchers can apply this vector to a wide range of mutation-related downstream tasking. Alternating, researchers can quickly observe the differential features of different groups(eg: case/control, high expression/low expression) by comparing those vector representation.

Conclusion:

This study presents a nucleotide representation model that interprets DNA sequences like reading a human sentence.

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Category: ___Bioinformatic tool_____

BIOLAKE : A RNA EXPRESSION ANALYSIS FRAMEWORK FOR PROSTATE CANCER BIOMARKER POWERED BY DATA LAKEHOUSE

Qiaowang Li[1], Yaser Gamallat [2,3,4], Reda Alhajj [1,5,6], Jon George Rokne[1] and Tarek A. Bismar[2,3,4,7]

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Introduction:

The field of biology is experiencing an exponential growth in available data. This growth has had the unfortunate effect that the extraction of the knowledge inherent in the data is not keeping pace with the data growth. The reason for this is that processing and extracting information from the data requires large volumes of processing guided by biologists. These biologists have to be conversant with computing and computational methods so that they can use the analysis tools effectively. Unfortunately, most biologists are not experts in the disciplines of mathematics, statistics or computing. The goal of BioLake is to provide users with a platform where they can perform a variety of bioinformatics analysis tasks in a minimalist interactive manner.

Methods:

BioLake is designed to be a framework where users interact with the underlying engines through a website designed with minimalist interaction concept. The mRNA data analysis consists of a) Differential Analysis - Heatmap, b) Differential Analysis - Volcano Plot, c) Expression Analysis - Clinical, d) Expression Analysis – Survival and e) Gene Set Enrichment Analysis.

Results:

This study presents an interactive web-based framework powered by a data lakehouse architecture that enables the enhancement and facilitation of raw data processing and analysis.

Conclusion:

BioLake provides a web-based framework for bioinformatic researchers, accessible at https://biolake.ucalgary.ca. The hope is that BioLake will allow researchers to focus more on scientific research rather than tool usage. BioLake does not apply manual inspection for any incoming data provided by the user to enhance its flexibility. And users can decide about the public visibility of provided data.

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Supervisor: Dr. Martin Hyrcza Category: Clinical Research

THE EYES HAVE IT: FACTORS INFLUENCING TEMPORAL ARTERY BIOPSY SENSITIVITY

Rhiannon Brett[1], William Trask[2], Martin Hyrcza[1]

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Introduction:
There is emergent disagreement in the literature regarding the broad implementation of temporal artery biopsy (TAB) in the assessment of suspected giant cell arteritis (GCA), as it has variable sensitivity based on many factors, and is limited by logistical constraints including barriers to access and the invasive nature of the test. However, TAB unequivocally remains the gold standard diagnostic for GCA. We sought to evaluate how various factors impact sensitivity including biopsy length, performing surgical specialty, referring specialty, and the relative contribution of individual clinical features.

Methods:
A retrospective chart review was performed of all TABs reported in our catchment area of 1.3 million people over a three year period (2019-2022). We classified the clinical presentation of query GCA patients based on the EULAR criteria, subclassifying into high and low pre-test probability based on the number of features present (high: two or more features). We subclassified specimens by specialty of performing surgeon; biopsy length and clinical pathologic concordance were documented. Analysis was performed by way of unpaired t-test and one-way ANOVA using a commercially available analytics suite (GraphPad Prism).

Results:
235 TABs from 230 patients (168 female) were performed. 54 biopsies were positive for GCA, 163 negative, and 14 were classified as indeterminate. Plastic Surgery performed 164 TABs and Ophthalmology performed 55. We found specimens performed by Ophthalmologists were, on average, longer with higher sensitivity than those performed by Plastic Surgeons (Ophthalmology 21.3 +/- 8.5mm, 80%; Plastic Surgery 14.7 +/- 6.1mm, 54%; $p< 0.0001$). On subgroup analysis, we identified visual symptoms to be strongest single predictor of a positive TAB: when present, TAB sensitivity was 78% vs 53% when absent.

Conclusion:
Our findings support the existing dogma that biopsy sensitivity is positively correlated with specimen length; in our institution, biopsy length differed significantly by surgical discipline. Our data suggest that visual symptoms are the most important symptom when predicting likely TAB outcome. The extent to which this accounts for the differential sensitivity by surgical discipline. These findings may help inform the relative weight to place on the EULAR criteria when evaluating patience and thereby optimize the cost-benefit ratio the clinical implementation of TAB.

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MOLECULAR PROFILE OF ATYPICAL LEYDIG CELL TUMORS

Diogo Caires[1], Yaser Gamallat[2], Asli Yilmaz[3], Fadi Brimo[4], Bob Argiropoulos[5], Tarek A. Bismar[6]

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Introduction:
Leydig cell tumors (LCTs) are the most common sex cord-stromal tumors of the testis. Although usually benign, a small minority of cases can be associated with a poor prognosis and metastasis. Histomorphological criteria, such as the Leydig cell tumor Scaled Score (LeSS) (which analyzes size, infiltrative margins, necrosis, vascular invasion, mitotic count, and nuclear atypia) have been recently developed to predict which tumors will behave in a malignant fashion ("atypical LCTs"). However, there is still a paucity of data in the literature with regards to their molecular differences. In the present study, we attempt to shed further light into the genomic characteristics of LCTs by performing whole copy number profiling in both benign and atypical/aggressive cases, as determined by the previously described LeSS.

Methods:
We performed whole copy number analysis using the Oncoscan platform by Thermofisher to compare the genomic profile of atypical (defined by the presence of any atypical features as described by the LeSS) vs benign Leydig cell tumors. Our sample consisted of 1 malignant (with biopsy-proven metastasis), 5 atypical, and 5 benign cases.

Results:
We found increased genomic instability in the malignant tumor and within 2 out of 5 (50%) atypical cases. One benign case revealed a likely pathogenic mutation in the Neurofibromatosis type 2 (*NF2*) gene, but all benign cases lacked genomic instability. Apart from the malignant case (which had metastatic spread to the scrotal skin), all remaining atypical cases did not reveal evidence of recurrence or metastatic spread.

Conclusion:
Copy number variations by itself are not sufficient to discriminate between cases that are benign versus those with malignant potential, without the use of histomorphogical parameters. Genomic instability was detected at a higher frequency in atypical and malignant cases compared to the benign ones and may represent an early step in malignant progression. Additional work needs to be performed to elucidate this relationship further. Currently, the presence of metastasis remains the only malignant criteria for Leydig cell tumors.

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SHORT-TERM FORMALIN FIXATION IMPAIRS ANTIGENICITY IN HUMAN LYMPH NODES FOR FLOW CYTOMETRY

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Introduction
One of the main obstacles that hinder the application of flow cytometry in patient care and clinical research is the strict requirement for fresh specimens. Despite continuous efforts made to understand the inhibitory mechanisms of formalin and rescue formalin-fixed specimens for flow cytometry in the past decades, the effects of short-term formalin fixation on cellular antigenicity are still surprisingly ill-illustrated. Herein, using flow cytometry, we revealed the changes induced by short-term formalin fixation in cell membrane integrity and cellular antigenicity on lymphocytes from human bowel lymph nodes.

Methods
A total of twenty-three (n=23) lymph nodes were harvested from fresh benign bowel specimens. The cell membrane integrity was determined among groups with different *ex vivo* periods. The cell membrane integrity of the lymph nodes with less than 48h *ex vivo* was further tested before and after 2h, 6h, 12h, 24h fixation with formalin respectively. The antigenicity of the lymph nodes with less than 48h *ex vivo* was also determined before and after 2h, 6h, 12h, 24h formalin fixation, with the applications of anti-CD19 and anti-CD45 antibodies in flow cytometry.

Results
Our results showed that the percentages of intact cells were significantly decreased when the *ex vivo* period exceeded 72 h. Moreover, the percentage of intact cells maintained over time after formalin fixation, whereas the antigenicity of lymphocytes started dropping upon formalin fixation and was completely lost till 6h after formalin fixation.

Conclusion
Fresh lymph nodes should be treated within 48h upon harvesting. Short-term formalin fixation impairs cellular antigenicity without changing cell membrane integrity in lymphocytes from human bowel lymph nodes. Our study reveals short-term time-dependent changes in antigenicity of human lymph nodes after formalin fixation, warranting a solid foundation for further establishing an antigen retrieval method to rescue formalin-fixed specimens for flow cytometric analysis.

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NON-HUMAN LEUKOCYTE ANTIGEN AUTOANTIBODIES ASSOCIATED WITH BIOPSY-PROVEN REJECTION AFTER KIDNEY TRANSPLANTATION

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Introduction:
Mismatched human leukocyte antigens (HLA) between a donor and recipient are the primary targets of antibody-mediated rejection (AMR) of transplanted organs. In addition, emerging evidence implicates a role for autoantibodies against non-HLA proteins in rejection, in the presence or absence of anti-HLA donor-specific antibodies (HLA-DSA). The technologies and study designs used to identify these non-HLA targets have been varied. Consequently, many questions remain, including the specificities relevant to each type of solid organ.

Methods:
A commercial bead assay offers the ability to test serum for antibodies against multiple non-HLA proteins at once. Vendor-established cutoffs for each target are based on median plus two standard deviations above the mean fluorescence intensity found in healthy male controls. We validated and adjusted the vendor cutoffs as needed against a local population of healthy males. Then, we tested pre-transplant and post-transplant sera associated with kidney transplant biopsies that indicated no rejection (NR), HLA-DSA$^-$ AMR or HLA-DSA$^+$ AMR.

Results:
In the NR and HLA-DSA$^-$ AMR groups, the cumulative burden of non-HLA autoantibodies decreased post-transplant, whereas it was not significantly different to pre-transplant in the HLA-DSA$^+$ AMR group. The number of non-HLA autoantibodies was significantly higher in HLA-DSA$^+$ compared to HLA-DSA$^-$ samples both pre- and post-transplant. Unexpectedly, the HLA-DSA$^-$ AMR group showed a significant decrease in non-HLA autoantibodies, both in terms cumulative burden and with respect to certain specificities, compared to NR samples. Comparison of NR and HLA-DSA$^+$ AMR patients identified pre-transplant PRKCH and post-transplant CXCL9 as autoantibodies significantly enriched in the latter group.

Conclusion:
These data support the synergistic activity of HLA-DSA and non-HLA autoantibodies in AMR. The increase and decrease in non-HLA autoantibodies found at the time of biopsy-proven rejection in HLA-DSA$^+$ and HLA-DSA$^-$ patients, respectively, compared to NR patients could represent overflow versus absorption into the inflamed kidney.

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Establishing a New Prognostic Prediction Module for Prostate Cancer Patients Under Active Surveillance Program Using a Combination of Genomic, Histological and Deep Learning Models

Joema Felipe Lima[1], Sima Seyedi[1], Yaser Gamallat[1], Norel Salut[1], Joshua Samsoondar[1], Biniyam Kahsay Mezgebo[1], Sunita Ghosh[2], Paul, Boutros[3] Adam Kinnaird[4] and Tarek A. Bismar[1] *

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Introduction:
This study investigates genetic, molecular alterations and mutation in prostate cancer as well as finding a histopathology signature and novel biomarkers in this disease.

Methods:
The study comprises two cohorts: patients with a Gleason Score (GS) of 3+3, with the case group exhibiting tumor progression to a GS greater than 3, and the control group remaining at GS 3+3 (active surveillance). In this study 350 cases in Alberta were assessed. 98 out of these were qualified for TST170 genomic sequencing. In order to find the histology signature, all the HE slides were assessed by the pathologist and were defined by pathology details (atrophy, inflammation and tumor extension), Moreover, 59 patient (11controls+48cases) slides underwent digital scanning to establish the deep learning models based on slides features.

Results:
As of today, DNA and RNA sequencing have been performed on 98 samples (34 cases, 54 controls), with plans to expand to 150 patients. More than 200 histology slides have been assessed to identify the histopathology signature of prostate cancer. Additionally, in terms of the deep learning model, more histology slides are continually being added to the project for investigation by AI to uncover meaningful patterns.

Conclusion:
The study aims to identify biomarkers, mutations, and pathology features associated with prostate cancer. By enhancing our understanding of the genomic and transcriptomic landscape, this research not only advances knowledge of the disease but also offers potential for early diagnostics and targeted therapies.

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Incidence of mutations in prostate cancer by DNA sequencing in relation to histopathological features.

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Introduction:
In the current study, we aimed to identify the most common mutations in 2 different cohorts of prostate cancer patients using Oncomine plus comprehensive sequencing Ion torrent platform and correlate the findings with histopathological features from these tumors.
Methods:
We investigated the incidence of mutations in prostate cancer (PCa), evaluated by genomic sequencing. Two different cohorts, comprised of men who had castrate resistant metastatic PCa, and non-metastatic PCa; and the second one, are samples from men who had locally advanced PCa (stage 3).
We then associated the frequency of mutations to pathological features, such as neuroendocrine (NE), poorly differentiated (PD), and acinar (AC) features; Gleason scores; and Gleason 4 pattern features, such as cribriform, vacuolized, fused and glomeruloid.
Results:
A total of 12 samples from locally advanced PCa cohort displayed TMPRSS2-ERG fusion in 2; ATM mutations in 6; BRCA1/2 in 5, and PALP2 in 2 samples. From this set of mutated samples, Gleason scores ranged from 4+4 (3); Gleason 4+3 (6); and Gleason 3+4 (3). Most cases had cribriform component (9), followed by 4 fused and 1 glomeruloid pattern. Among the 20 non mutated samples from the same cohort, Gleason scores ranged from 4+4 (4); Gleason 4+3 (8) and Gleason 3+4 (8). Here again, most frequent component was cribriform (16), followed by fused (3), vacuolized (3) and glomeruloid (3). Additionally, a total of 12 samples from the castrate resistant metastatic and non-metastatic PCa were mutated, and displayed BRCA1/2 (6); ATM (3), CDK12 (1) and RAD50 (1). From the mutated cases, 4 PCa had NE, 4 PD and 4 AC features.
Conclusion:

We described a set of mutations that were present in PCa, from two different cohorts and their relationship with histopathological features of these PCa. Further data analysis on this dataset will help us to characterize these features better.

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Supervisor: Dr. Amy Bromley Category: Clinical Research

EXPLORING THE LEARNING EXPERIENCES OF EARLY PATHOLOGY RESIDENCY TRAINING THROUGH THE LENS OF REALIST INQUIRY

Dr. Kyo Farrington[1], Dr. Rachel Ellaway[2], Dr. Amy Bromley[3]

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Introduction:

There is a recurring and challenging knowledge gap transitioning residents experience in Diagnostic and Molecular Pathology (DaMP) and Diagnostic Clinical Pathology (DCP). The cause of this seems to be minimal pathology-specific training in medical school, thereby limiting significant opportunities for pathology-specific knowledge acquisition and application prior to residency.

A new curriculum model was introduced during the Foundations of Discipline (FoD) stage of residency to address this gap. While the efficacy of the module was supported by casual observations and feedback from participants, the unique challenges faced by early pathology residency trainees was still unclear. We therefore conducted a study: 1) to explore and characterize the learning challenges faced by early pathology residency trainees, and 2) to test our hypothesis that semi-structured protected learning time in concert with graduated workplace-based training are key components of early pathology residency training and may attenuate learning challenges.

Methods:

A critical realist methodology was used to identify the mechanisms that make the transition to pathology residency training so challenging. We designed the study around the following research questions: 1) "*What are the perceived learning challenges faced by early pathology residency trainees?"* and 2) "*What are the components of effective learning in early pathology residency training?*" Data were collected between January-April 2024 through an anonymous survey administered to DaMP/DCP residents who had completed FoD with optional follow-up semi-structured interviews. De-identified transcripts were analyzed using thematic analysis techniques including identifying "context-mechanism-outcome" configurations from which middle range theories were developed that may be used to guide residency program changes and innovations.

Results:

Preliminary analysis has highlighted various learning challenges in early pathology residency training, predominantly related to the volume of novel information and experiences encountered early in training. Aspects of the novel curriculum have had a positive impact on the learner experience, albeit, with room for iterative optimization.

Conclusion:

While further synthesis and integration of our findings into program theory is required, preliminary realist evaluation of the early pathology resident experience has enabled exploration and characterization of the learning challenges faced by early trainees and has highlighted multiple avenues that can be targeted to attenuate these challenges.

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VALIDATION OF PROGNOSTIC STRATIFICATION BY NOVEL RISK GROUPS FOR UTERINE LEIOMYOSARCOMA

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Background: Uterine leiomyosarcomas (LMS) are lethal gynecologic mesenchymal malignancies arising from the myometrium. They represent the most common type of uterine sarcomas (~ 80% of all uterine sarcomas), accounting for 5% of all uterine cancers. While surgery can be curative for about 50% of patients diagnosed at an early stage (stage I), advanced or recurrent disease is minimally responsive to current standard adjuvant treatments. Recurrent somatic mutations in TP53, ATRX, MED12, PTEN, MEN1 and RB1 genes have been reported in LMS. Chapel et al. [PMID: 35121810] recently reported that morphological features can be used for risk stratification of stage I LMS. We aimed to apply their criteria in our population.

Methods: In the present retrospective cohort study, we evaluated the key morphologic features in 34 uterine LMS cases diagnosed between 2016-2022 at Foothills Medical Center, Calgary, AB. Gross morphologic and key histologic features, including tumor size, tumor myometrial interface, cervical and adnexal involvement, and nuclear grade, were recorded. Mitotic count per 10 high power fields was counted in hotspot region after screening the entire tumor. Predominant morphology (spindle, epithelioid or myxoid) was recorded as per the recent WHO classification definitions. Nuclear grade I to III was defined based on degree of cytologic atypia, overt nuclear pleomorphism, cytoplasmic eosinophilia and fascicular architecture. Risk scores were calculated according to Chapel et al. based on presence of necrosis, mitotic rate>25/10HPF, atypical mitosis, lymph vascular invasion, and serosal abutment. Risk groups were calculated where possible.

Results: The overall 5-year survival time for the 34 patients with uterine LMS was 44.5%. Tumor size, FIGO stage, adnexal involvement, and nuclear grade were significantly associated with status at last follow up (Table). In univariate Kaplan-Meier survival analysis, none of the individual features proposed by Chapel et al. were significantly associated with survival in our smaller cohort. However, the combined risk groups stratified our cohort regarding survival (log rank, p-value = 0.030, Figure).

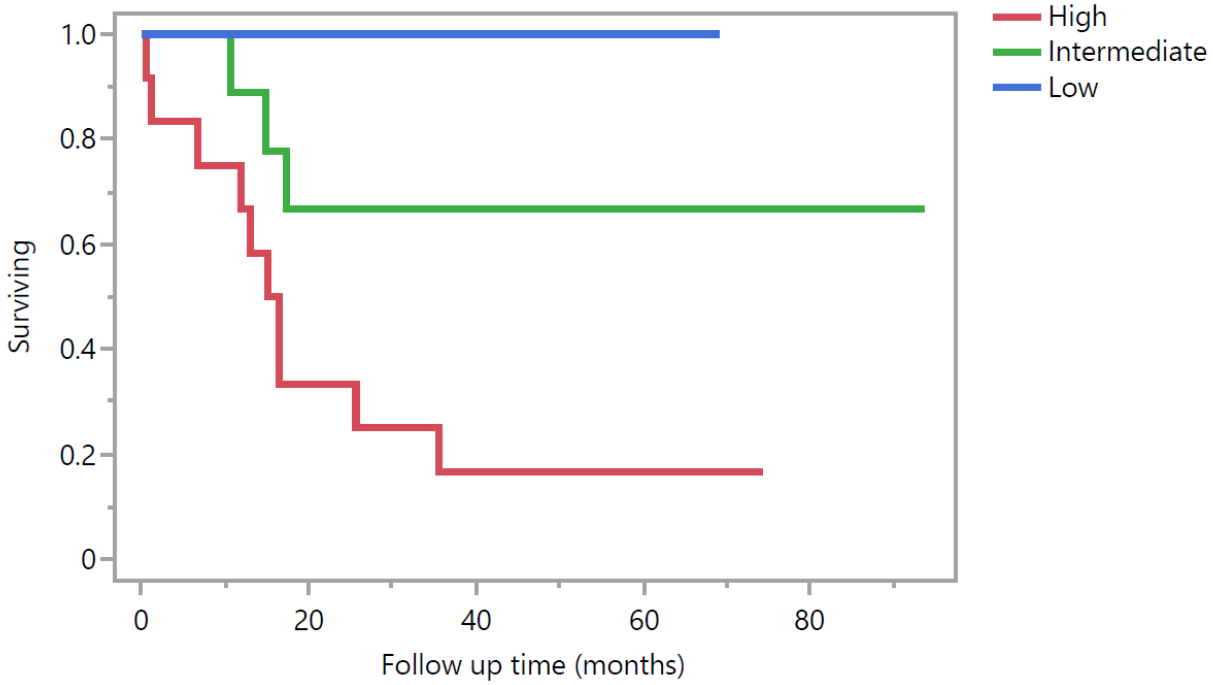
Conclusion: The novel risk groups proposed by Chapel et al. assessed on primary uterine LMS stratified patients according to risk of death without taking stage into account. Although our study is limited by low case number, this provides indirect validation of the findings by Chapel et al. Biomarker studies are ongoing.

Table

		ALIVE (n=17)		DEAD (n=17)		P-value
	Feature	**MEAN**	**SE/%**	**MEAN**	**SE/%**	
	Age	54.5	2.6	59.3	2.6	0.21
	Tumor Size	12.0	1.8	17.5	1.7	0.032
	Mitotic count	25.5	6.3	41.4	6.4	0.086
FIGO Stage	**Confined (stage I)**	8	100	9	34.6	0.0012

	Beyond (stage II-IV)	0	0	17	65.4	
MORPHOLOGIC FEATURES						
Necrosis (tumor)	**Indeterminate**	0	0	1	6	0.13
	Absent	3	18	0	0	
	Present	14	82	16	94	
Mitosis (>25/10HPF)	**Absent**	10	59	6	38	0.22
	Present	7	41	10	52	
Atypical Mitoses	**Absent**	7	41	4	24	0.27
	Present	10	59	13	76	
Lymph vascular invasion	**Indeterminate**	1	6	2	12	0.16
	Absent	12	71	6	35	
	Present	4	24	8	47	
Serosal abutment	**Indeterminate**	6	35	4	24	0.15
	Absent	9	53	6	35	
	Present	2	12	7	41	
Chapel risk score group	**Low (0-2)**	2	18	0	0	0.012
	Intermediate (3-5)	7	64	3	23	
	High (6-13)	2	18	10	77	

Figure



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A centralized, provincial approach to gene fusion detection

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Introduction: Gene fusions feature prominently as oncogenic drivers in epithelial and stromal neoplasia. Headed by the South Zone molecular core, in April 2022, Alberta Precision Labs (APL) onboarded two cost-effective, semi-heuristic RNA-based gene fusion panels: the Archer Lung and Pan Solid Tumor FusionPlex. These panels were paired with Ion Torrent sequencing technology. Here, we describe a preliminary provincial experience.

Methods: Approximately 800 samples were sequenced in the first year of Archer fusion panel introduction by APL. In retrospective review, we merged surgical pathology, molecular, and clinical variables for n=140 (18%) of the initial portion of this cohort to produce a descriptive summary. We then used contingency coefficient analysis and multivariable linear regression to quantify associations.

Results: The majority of cases were from the Calgary zone (70%), with an equal distribution by patient sex (female 52%), and a median age of 55 (IQR: 40-70). The most prevalent tumor types were lung (34%), soft tissue (29%), and brain (13%); the majority of samples submitted were non-metastatic (contingency coefficient 0.958, *P*=3.05e-174). Biopsies constituted the majority of cases (55%) and macro-dissection was performed 44% of the time to improve tumor yield. Median tumor percent was 70% (IQR 40-80%), with a median RNA yield of 92.6 ng/ul (IQR 37.0-218.0 ng/ul). The median preseq Ct value, a measure of quality, was 25.27 (IQR 24.05-27.65), while the median average unique start sites per GSP2 primer per library was 88.88 (IQR 48.48-112.12). Only a lower preseq Ct value was predictive of improved sequencing quality (-8.88; 95%CI -5.44, -12.32; *P*=2.11e-6) in multivariable regression with specimen type, tumor percentage, macro-dissection, and RNA concentration. Seven cases (5%) were cancelled prior to sequencing, while 19 cases (14%) failed sequencing. Thirty-nine fusions were detected; receptor tyrosine kinases featured most prominently (46%), while the most frequently reported fusion was SS18::SSX1-4 (n=4). Disease defining fusions were identified for 74% of cases harbouring a fusion.

Conclusion: APL has successfully implemented centralized fusion detection platforms that serve to support histomorphological diagnoses for nearly three in four patients in which a fusion driver is identified.

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Fusion partner agnostic approaches improve detection of targetable gene fusions in thyroid cancers

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Introduction: Rearrangements serve as oncogenic drivers for roughly one-in-five thyroid tumors. This underscores the importance of a comprehensive molecular approach that extends beyond assessment of single nucleotide variants (SNV). Receptor tyrosine kinases (RTK) feature prevalently within the described gene fusions in thyroid entities. RTKs are highly indiscriminate, both in their fusion partners and the underlying genomic mechanism of rearrangement, which can fall below the detection limit of conventional diagnostic approaches. As a consequence, targeted multiplex amplicon sequencing has emerged as a preferred method for characterizing thyroid neoplasia. However, such approaches require a priori knowledge of fusion partners and/or breakpoints. Therefore, it was hypothesized that gene partner and/or breakpoint agnostic approaches might impart improved clinical sensitivity.

Methods: In a primary sample across four marketed thyroid sequencing panels (ThyroSPEC, Oncomine Focus Assay (OFA), Illumina TruSight Fusion Panel (IFP), and Archer FusionPlex CLT), we performed an in silico analysis on n=20 unpublished cases of fusion-driven thyroid carcinomas identified using retrospective FusionPlex sequencing, between 2022-2023. To supplement this data, we identified 16 publications reporting at least five thyroid cancer fusion-driven cases, however, 13/16 (81%) were excluded due to lack of transcript or genomic reference IDs and 1/16 (6%) was excluded due to utilization of a targeted multiplex amplicon approach, for a final total of n=168. Sensitivity analysis was performed through stratification via the five most common RTK fusion partners/families: RET, NTRK1-3, BRAF, ALK, and MET. Proportions tests were used to evaluate significance, with multiple hypothesis correction.

Results: We observed that FusionPlex could identify 100% of the 168 described fusions, compared to a detection frequency of 57%, 60%, and 65% for ThyroSpec, IFP, and OFA, respectively (P=1.11e-20). Subset analysis revealed similar significance for BRAF (P=7.36e-19), NTRK1-3 (P=3.69e-7), and MET (P=0.023), however RET and ALK did not reach significance.

Conclusion: Collectively, these findings demonstrate the critical importance of panel selection for identifying fusion-driven thyroid cancers and that a gene partner and/or breakpoint-agnostic approach to fusion discovery has improved clinical sensitivity.

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CREATION OF A QUALITY ASSESSMENT FRAMEWORK: A CASE STUDY OF THE CALGARY AUTOPSY SERVICE

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Introduction:
To support an environment of continuous quality improvement a vital starting point is to develop a robust methodology to accurately assess the current state of a service. This project sought to create a comprehensive assessment framework to collect both quantitative and qualitative data for a database that would be used to gain a broader understanding of a lab service and identify opportunities for quality improvement. As a case study, the hospital-based autopsy service at the Foothills Medical Centre (FMC) (Calgary, Alberta) was selected. A comprehensive audit of this service has not been previously completed.

Methods:
Internal and external expert consultation was undertaken to create a framework for data collection. Four major areas and relevant metrics were identified. The areas included utilization, quality, workload, and education/research. Metrics were established to move data collection beyond superficial criteria. In total over 55 different data metrics were identified. All autopsies performed at the FMC from February 1, 2017 to July 1, 2021 were included and reviewed to identify these metrics

Ethics approval: University of Calgary Conjoint Health Ethics Board (REB21-1408).

Results:
A robust framework was developed to retrospectively assess the autopsy service. During the study period a total of 867 autopsies were performed, producing more than 2400 reports and providing more than 100,000 datapoints for the database. The data was collected in a way that can be interrogated to assess for trends and response to quality improvement initiatives.

Conclusion:
In conclusion, the creation of a comprehensive quality assurance framework and subsequent database will provide valuable data-driven insight into the Calgary Autopsy Service for future quality improvement, workload analysis, and educational reviews. Additionally, these methods and metrics can be generalized for transferability for a more vigorous assessment of other lab services.

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COMMON PARKINSON'S DISEASE THERAPY LEADS TO FALSELY LOW URINE AND PLASMA CREATININE

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Introduction:

A 79-year-old male outpatient with no history of kidney disease presented to the laboratory with an albumin-to-creatinine ratio (ACR) ordered on a urine specimen. Upon initial analysis, the urine creatinine results produced a less than test value. The study goal was to determine the underlying cause of the undetectable urine creatinine and assess the patient impact of falsely low creatinine results in urine and plasma.

Methods:

Our laboratory measures serum and urine creatinine on the Roche Diagnostics cobas c701 via coupled enzymatic reactions. A Siemens Healthineers Atellica CH analyzer using a modified Jaffe method was used as a comparison creatinine assay. Creatinine was measured by both methods on the initially flagged urine sample, as well as a follow up urine and plasma samples collected three weeks later.

Results:

The initial urine creatinine measured on Roche cobas c701 was <0.1mmol/L, which flagged the specimen for further investigation. A review of Roche cobas c701 reaction traces showed an abnormal pattern, indicating the presence of an interfering substance. Urine creatinine was detectable on the Siemens Atellica, which allowed the calculation of ACR. Review of the patient's medical record showed long-term combination therapy of Levodopa and Carbidopa to treat Parkinson's Disease. Levodopa causes a negative interference at therapeutic concentrations in the Roche cobas c701 creatinine assay. In contrast, Levodopa causes a positive interference on the Siemens Atellica only at supraphysiological concentrations. Follow up urine and serum samples showed the persistence of falsely low creatinine values in both sample types on the cobas c701 compared to the Atellica. The falsely decreased plasma and urine creatinine resulted in a 36% and 150% false elevation of eGFR and ACR, respectively.

Conclusion:

Levodopa is commonly prescribed for Parkinson's disease and can cause falsely decreased creatinine results in urine and plasma samples when measured by peroxidase-based enzymatic methods.

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THE EFFECT OF HEMOLYSIS ON POTASSIUM MEASUREMENT IN A COMMUNITY SETTING: THE MARCHING ERROR

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Introduction:
Potassium is one of the most sensitive analytes to in-vitro hemolysis. The goal of this study was to determine a clinically acceptable HI cut-off for potassium at our community reference laboratory, relying on patient data from routine laboratory operation.

Methods:
Plasma venous potassium and HI test results from community patients measured on Roche cobas 8000 ISE and c701 modules between September 2022 to December 2023 were extracted from the Roche Infinity Middleware (n=1,101,897). Data was divided into groups based on HI which ranged from 0 to 300. Mean, standard deviation, absolute and percent change of potassium for each HI group was calculated. Data were analyzed in Rstudio and Microsoft Excel.

Results:
The vast majority of potassium specimens (83.8%) were stratified into the HI base group (HI ≤10) and 96.8 % had an HI ≤ 20, the cut-off recommended by Roche. The mean potassium level for each HI group increased as the degree of hemolysis in the specimens became more elevated. Depending on the employed total allowable error limit, clinically significant shifts in mean potassium levels were observed at HI bin 41-50 (RCPA: ΔK>0.2 mmol/L) or HI bin 101-150 (EFLM-derived reference change value: ΔK>10.1% and CLIA: ΔK>0.5mmol/L). The shift in patient potassium values seen as HI increased led to a change in critical results reporting frequencies. In the group of HI≤10, 0.06% and 0.07% of results had low (<2.6 mmol/L) or high (>6.2 mmol/L) critical potassium values, respectively. When HI was 101-300, 0.00% and 15.7% of results had low or high critical potassium values. Linear regression of ΔK plotted vs HI produced the equation ΔK=0.0051xHI, validating the equation of Martinez-Morillo and Alvarez published previously using paired hemolyzed/non-hemolyzed potassium laboratory data.

Conclusion:
In community patients, in-vitro hemolysis produced clinically significant errors at HI>40. When HI>100, mean ΔKs of 0.5-1.20 mmol/L were seen in our population. This shift in patient values as a function of hemolysis led to a change in critical result reporting, suggesting the generation of both factious critically high potassium results and the masking of critically low potassium results.

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REDUCING INAPPROPRIATE FECAL IMMUNOCHEMICAL TESTS

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Introduction:
Colorectal cancer is the third most common cancer worldwide. The risk of colorectal cancer increases with age and predominantly affects individuals over the age of 50. Regular screening is crucial for early detection and prevention of colorectal cancer. Pre-cancerous polyps and colorectal cancers can bleed into the gastrointestinal tract. While the presence of blood in the stool cannot always be seen visually, it can be detected by the Fecal Immunochemical Test (FIT), currently the most sensitive method for directly measuring hemoglobin in this specimen type. In our province, FIT is recommended for testing average-risk asymptomatic patients aged 50 to 74 every 1-2 years. Patients aged 40 to 49 or 75 to 84 can be tested if they meet specific criteria. Like many other tests in the lab, FIT can be used inappropriately. The focus of this study was to examine the utilization of FIT in our institution.

Methods:
Data were collected from our institution's laboratory information system for the FIT performed on-site from July 1st to December 31st, 2023.

Results:
We performed 87,818 FIT tests during the 6-month period with an overall positive rate of 7.1%. Of the total tests performed, 79,601 tests (90.6%) were for the patients aged 50-74 years, the recommended age range for colorectal cancer screening in Alberta. 7,314 tests (8.3%) were for patients aged 40-49 or 75-84 years, and 903 tests (1%) were for patients aged under 40 or above 84 years. Each year, we performed approximately 1,800 FIT tests for patients outside the accepted screening age, with an estimated cost of 30,000 - 60,000 CAD.

Conclusion:
Our results showed a significant number of FITs were ordered inappropriately by healthcare providers. Considering the relatively high cost of FIT kits, labor, reagents, etc., the overall cost per reportable is significant. Thus, we have started engaging healthcare providers involved in FIT testing to strategize approaches to improve utilization.

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Evaluation of Microtomy Sectioning Protocol for Breast Needle Biopsies in Alberta Precision Laboratory using Unstained Slides.

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Introduction:
To our knowledge, there is currently no universally accepted protocol for the upfront cutting of unstained slides for breast biopsy specimens. However, it is a standard practice followed by the Alberta Precision Laboratory. The objective of this study is to assess the frequency of using upfront unstained slides in the diagnostic evaluation of core needle breast biopsies.

Methods:
We conducted a review of all breast needle biopsies that were submitted for microtomy sectioning at Alberta Precision Laboratory in Calgary from January to December 2021. All breast biopsies were processed using the standard breast biopsy protocol, which included one H&E, two additional H&E levels, and 6 unstained slides initially. We then determined if the unstained slides were utilized in the diagnostic workup of core needle breast biopsies.

Results:
Results: A total of 3,202 breast biopsy cases were received from January to December 2021. Majority of cases, 1,937 (60.49%) were diagnosed as benign, 1,189 (37.13%) as malignant, and 61 (1.91%) as atypical (e.g. ADH, atypical papillary neoplasms, etc).

There were 25,366 unstained slides produced, out of which 9,488 were used for lesions requiring immunohistochemistry and/or breast biomarkers. The utilization rate varied depending on the type of specimen. For simple benign cases requiring workup (e.g., demonstrating myoepithelium), a low utilization rate (12%) was noted. In contrast, a higher rate of utilization rate (78%) was noted for malignant lesions which often require reflex biomarker determination and/or further diagnostic work-up. Atypical lesions (e.g., ADH, atypical papillary neoplasms, etc) and those with indeterminate histology that require resection for definitive diagnosis have a 44% and 64% rate of utilization, respectively. The overall rate of utilization is 37%.

Conclusion:
Conclusion: Our study outlines the standard protocol for needle core breast biopsy at Alberta Precision Laboratories. The findings suggest that there is a low overall utilization rate for upfront unstained slides with an overall rate of 37%. The greatest utility of upfront unstained slides is noted in malignant cases that often require breast biomarker profiling.

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A Case Report of Testicular Mammary type Myofibroblastoma

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Introduction:
Mammary-type myofibroblastoma is a rare benign tumor of myofibroblastic cells. We report a case of mammary-type myofibroblastoma in a 38-year-old man presenting with a scrotal mass.

Case Presentation:
A 38 y.o man presented with a 6.8 cm left scrotal mass on ultrasound. Intra-operatively, the scrotal mass was found arising from the tunica vaginalis with the testis being unremarkable. Grossly, the tumor showed a well-circumscribed, homogenous lipomatous surface. Microscopic sections of the neoplasm showed a moderately cellular spindle cell neoplasm with a fascicular growth pattern. The tumor cells demonstrated short tapering nuclei with pale eosinophilic cytoplasm on a background of hyaline collagenous stroma containing scattered mast cells admixed with adipocytes. There was no significant atypia, mitosis or necrosis. The lesional spindle cells were positive for CD34 and desmin and negative for SMA, MDM2 and CDK4. The staining for Rb is positive (normal/retained) and the whole copy number analysis using the Oncoscan platform by Thermofisher showed LOH of chromosome 13q21 and loss of chromosome 5p15.

Conclusion:
Mammary-type myofibroblastoma arising from the tunica vaginalis is a rare benign tumor. A review of current case reports and case series have not mentioned this type of tumor arising from this site. In a series of nine cases of soft-tissue mammary-type myofibroblastoma reported by McMenamin et al. in 2001, seven cases occurred in males in the perineal distribution and one case presented as a paratesticular mass. These tumors were hypothesized to arise along the embryonic mammary ridges from the axilla to the mid-groin. However, mammary-type myofibroblastomas have been reported in other locations distal to the mammary ridge such as the liver, seminal vesicle, abdominal wall, big toe and in our case the tunica vaginalis. 92% of myofibroblastoma will show loss of nuclear expression of Rb1 on IHC. Our case showed an intact/retained Rb1 expression on IHC. Whole copy number analysis using the Oncoscan platform by Thermofisher was performed to analyze the genomic profile of the tumor which identified LOH on chromosome 13q21 and loss of chromosome 5p15. The loss of chromosome 5p15 is associated with Cri du chat syndrome, however this will be confirmed on clinical follow-up.

Histopathological examination and ancillary studies play a critical role in establishing the diagnosis of mammary-type myofibroblastomas to avoid overtreatment. The differential diagnosis can be challenging due to overlapping features with both benign and malignant neoplasms. These tumors have an indolent, benign course with rare local recurrences.

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AUTOPSY EDUCATION: REVIEW OF A LARGE CANADIAN ACADEMIC CENTRE

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Introduction:

Autopsy is unique due to the breadth of entities that can be encountered, many of which are not found in routine surgical pathology. Introduction of Competency Based Medical Education raised questions as to which entities residents should be competent with and how to best assess competency. There is little published data regarding which entities residents routinely encounter. Based on Canadian statistics and our unpublished data, the most important natural causes of death for residents to be competent in include atherosclerotic cardiovascular disease (ASCVD), sepsis/infection, malignancy, primary pulmonary disease, post-operative complications, and renal failure. We sought to gather comprehensive data to identify trends and gaps in the resident autopsy experience to enable evidence-based quality improvement of residency education.

Methods:

A retrospective review of the Calgary Autopsy Service was conducted using methods described in “Creation of a Quality Assessment Framework: Case Study of the Calgary Autopsy Service”, House et al. (unpublished). The study period spans 4 academic years (July 1, 2017 to June 30, 2021). All hospital-based cases with a primary pathology resident (who performed the autopsy and wrote the report) on their core autopsy rotations were included. Pediatric, central nervous system only, and on-call autopsies were excluded.

Ethics approval: University of Calgary Conjoint Health Ethics Board (REB21-1408).

Results:

432 out of the 772 autopsies performed fit our selection criteria. On average 82% (± 6.2%) of autopsy cases per academic year involved a resident on their core rotation. On average, residents (n=26) completed 16.6 (± 4.4) cases during these rotations. There was major variability between residents regarding their exposure to all key entities except ASCVD. Only one resident was directly exposed to all pertinent entities.

Conclusion:

While residents are comparable to the national average for hospital autopsies performed by a junior resident, we have identified gaps in the types of cases residents are involved in. Given these gaps, we must ensure a robust formal curriculum to ensure foundational knowledge in all pertinent pathologies for all residents. Additionally, complexity such as patient safety and health equity must also be incorporated to ensure competence in adult medical autopsy.

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EVIDENCE-BASED TEST UTILIZATION: TEST ORDERING PATTERNS IN FUNCTIONAL MEDICINE AND COMMUNITY CLINICS, AND IMPLICATIONS FOR LABORATORY STEWARDSHIP

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Introduction:
Functional Medicine Physicians (FMPs) use a holistic approach to identify and address "the root cause of a disease". These physicians routinely order laboratory tests that are not supported by clinical suspicion and practice guidelines (i.e., Centers for Disease Control and Prevention Laboratory Medicine Best Practices, and local diagnostic algorithms) and are unnecessarily duplicative, with inappropriate testing patterns. Ordering unnecessary tests does not improve diagnosis; indeed, 5% of disease-free patients falsely test positive for a given diagnostic test. Test overutilization increases the rate of false positives, leading to unnecessary medical follow-ups, compromised patient care, and financial constraints on the healthcare system. The objective of this study is to: 1) Compare test ordering patterns between FMPs and General Practitioners (GPs) to identify disparities and opportunities for laboratory stewardship improvements. 2) Analyze the potential factors impacting test utilization in areas served by FMPs, 3) investigate the proper approach to improve test utilization.

Methods:
Using our laboratory's information system, we conducted a retrospective study spanning from January 2022 to December 2022 to assess testing patterns by 15 FMPs in Southern Alberta, comparing them to those of randomly selected community GPs within the same geographical region. A query was executed to retrieve data on the top 50 chemistry tests ordered by functional medicine clinics and community clinics. These tests were further categorized into six groups: general chemistry, endocrinology, immunology, trace elements, toxic metals, and vitamins. Data analysis included determining the geographical locations of functional medicine clinics and assessing the socioeconomic status of their patient populations. Additionally, the percentage of abnormal test results were calculated for both functional medicine clinic and community clinics. Furthermore, the average cost of tests per patient, per physician, and per clinic was computed to evaluate the financial implications of testing practices.

Results:
Our preliminary results showed significant variability in test ordering practices among FMPs, with potential implications for patient care and healthcare costs. Most tests ordered by FMPs were normal (94%), suggesting a need for further scrutiny regarding the

necessity and appropriateness of these tests. A notable trend observed was the disproportionate preference of FMPs for expensive tests, including hormones, vitamins, and trace metals. Our analysis reveals that the cost per reportable test from FMPs was four times higher compared to the tests ordered by GPs. Furthermore, on average, functional medicine clinics in urban areas ordered around 60% more tests annually compared to their rural counterparts.

Conclusion:

Our preliminary findings clearly show a need to monitor the testing behavior more carefully by the functional medicine clinics. The data from this study will be shared with the physicians practicing functional medicine in Alberta. Strategies to improve the test utilization and patient care include educating the functional medicine doctors and provincial healthcare authorities about the appropriate test utilization and the impact of inappropriate use of lab on patient care. In addition, using algorithms for test ordering will be used if the pattern of inappropriate testing continues.

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EARLY DETECTION OF ACUTE GRAFT VERSUS HOST DISEASE BY ASSESSMENT OF THE IMMUNITY RELATED TRANSCRIPTOME

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Introduction:
Graft versus host disease (GvHD) is the most common cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT). Acute GvHD (aGvHD) which typically manifests early after transplant mainly affects the skin, gastrointestinal track, and the liver with different grades of severity. aGvHD grade 2-4 is routinely treated after the development of signs and symptoms resulting in poor response in ~50% of patients. If an early and accurate prediction of ensuing aGVHD is possible, then the high-risk patients can be identified and treated preemptively. Here we present potential immune transcriptome markers that can identify patients at high risk of aGvHD before the signs and symptoms develop.

Methods:
Immunity-related transcriptome profiles at Day14 post-transplant of patients diagnosed with aGVHD grade 2-4 (n=13) were compared with patients with no aGVHD (n=17). Median day of diagnosis of aGvHD gr2-4 was Day34. All patients received myeloablative conditioning therapy along with anti-thymocyte globulin prophylaxis before transplant. Total RNA was extracted from cryopreserved peripheral blood mononuclear cells at Day14 post-HCT. Gene expression profiling of 579 immunity-related genes was conducted using NanoString Technology.

Results:
Patients diagnosed with aGvHD gr2-4 were found to have significantly upregulated expression (Benjamini Hochberg Pvalue (BHP) <0.05) of cytotoxic T-cell related surface markers and signaling molecules at Day14 post-transplant before the occurrence of signs and symptoms of aGvHD. The expression pattern of a panel of 12 significantly upregulated genes namely CD3D(BHP=0.003), CD3E(BHP=0.001), CD8A(BHP<0.001), CD7(BHP=0.002), STAT4(BHP<0.001),LCK(BHP=0.001), SH2D1A(BHP=0.003), IKZF3(BHP<0.001), KLRK1(BHP=0.001), KLRB1(BHP<0.001), ZAP70(BHP<0.001), CD247(BHP<0.001) was used to create a gene score for the prediction of aGvHD. A gene score of >5 was able to identify patients at high risk of aGvHD gr2-4 with a sensitivity of 92% and specificity of 88%.

Conclusion:
The immunity related transcriptomic panel of 12 genes identified in this study has the potential to differentiate patients at high risk of aGvHD gr2-4 with high sensitivity and specificity with a technique that can be easily translated into clinical practice. These results can pave the way towards development of preemptive therapy for aGvHD thereby reducing the risk of HCT as a curative therapy for leukemia.

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DIFFERENTIAL T CELL RECONSTITUTION AT THREE MONTHS POST HCT INFLUENCES THE IDENTIFICATION OF A LACK OF GVL REACTION.

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Introduction:
Relapse of leukemia is the major cause of mortality after allogeneic hematopoietic stem cell transplantation (HCT) representing a lack of graft versus leukemia (GvL) allo-immune reaction. There is a need for highly sensitive and specific markers that can identify patients at high risk of relapse. In this study, we demonstrate that the recovery of Tcell related genes at the transcriptomic level is an important factor influencing the identification of transcriptome markers for prediction of relapse.

Methods:
Immunity-related transcriptome profiles at 3-months post HCT of patients diagnosed with acute leukemias were studied (n=48). All patients received myeloablative conditioning therapy along with anti-thymocyte globulin prophylaxis. Total RNA was extracted from cryopreserved peripheral blood mononuclear cells at 3-month post-HCT. Gene expression profiling of 579 immunity-related genes was conducted using NanoString Technology. Tcell counts were determined by multicolor flow cytometry.

Results:
Unsupervised K-means clustering of the patient population based on the recovery of the immunity related transcriptome led to the stratification of the patients into two clusters A and B. Cluster B was found to have a significant higher expression of Tcell related genes as well as Tcell counts compared to cluster A. Differential gene expression analysis between patients that relapse vs don't relapse was separately conducted in the two clusters A and B. Among Cluster A (global low Tcell gene expression), patients that relapse had significantly downregulated expression of 13 genes representing Tcell signaling and exhaustion markers. The top 8 differentially expressed genes were able to identify patients at high risk of relapse with 100% sensitivity and specificity. Whereas among patients in cluster B with global high Tcell gene expression, the markers to predict relapse could not be found.

Conclusion:
Relapse specific transcriptomic signature at 3-months in HCT recipients with AML and ALL could be easily identified in the cluster of patients with the lower expression of T cell related genes whereas such signature could not be identified in the cluster of patients with higher recovery of Tcells. Thus, Tcell reconstitution at the cellular and transcriptomic level after HCT can have a significant influence on the discovery of markers indicating relapse.

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Identification of Amyloidogenic Proteins in FFPE tissues using Targeted LC-MS/MS

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Introduction:

Amyloidosis consists of a large group of diseases caused by deposition of misfolded protein aggregates in a variety of tissues causing organ damage and a variety of clinical syndromes. More than thirty unique amyloidogenic proteins have been identified giving rise to different forms of amyloidosis with unique treatment options. Diagnosis and subsequent treatment is dependent on identification and accurate typing of the amyloid plaque. Therefore, proteomics techniques utilizing high-resolution liquid chromatography-mass spectrometry (LC-HRMS) were developed at the Mayo Clinic. These methods are costly, time-consuming, and employ finicky instrumentation such as laser capture microdissection systems not generally available to clinical laboratories in Canada. This work presents a low(er)-cost, targeted mass spectrometry approach to identify amyloidogenic proteins from formalin-fixed paraffin-embedded (FFPE) tissue sections. This method will provide more timely identification of amyloid proteins at a lower cost, promoting better patient care in Alberta.

Methods:

Sections of fat pad aspirates with amyloid plaques which had previously undergone identification by Mayo Clinic were obtained from de-identified patients. Amyloid proteins were extracted from these tissues following established protocols. Proteins were digested with trypsin, and peptides were subject to a targeted LC-MS analysis using predicted precursor-product ion transitions determined using the proteomic software Skyline. Each protein was considered ‘detected’ by the presence of at least three tryptic peptides in the LC-MS. Accuracy of the targeted in-house method was then evaluated against the results previously obtained from Mayo Clinic.

Results:

Initial analysis has permitted identification of multiple unique peptides from immunoglobulin kappa and lambda light chains from AL amyloid positive FFPE tissues. Additionally, general amyloidogenic proteins were also identified, including apolipoprotein A-4, apolipoprotein E, and serum amyloid A which are required for deposition of amyloid proteins in tissues. Further work is underway to define the quality of the targeted LC-MS approach by assessing requirements for minimum tissue quantity for analysis as well as detection limits for each protein.

Conclusion:

This work presents a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) method for detection of amyloidogenic proteins. Initial investigations demonstrate our method is able to identify proteins in AL amyloidosis, with further work to be conducted. This work presents a straight-forward, accurate and cost-effective method for determining amyloid type that could be applied to other, rare amyloid types.

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NASOPHARYNGEAL TUBARIAL GLANDS ARE MINOR SALIVARY GLANDS

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Background: Tubarial glands are putative major salivary glands in the nasopharynx overlaying the torus tubarius and Eustachian tube. Their existence has been proposed in 2021 by Valstar et. al. (Radiotherapy Oncology 2021 vol.154 p.292-8) based on the observation of a distinct area of prostate-specific membrane antigen ligand (PSMA)-positive tissue in the nasopharynx, with similar ligand uptake characteristics to major salivary glands, using positron emission tomography/computed tomography (PET/CT) in patients with prostate cancer. To date, there has been no anatomical or histological confirmation of the presence of major salivary glands in this location. Thus, the primary objective of this study was to define the morphology of tubarial glands and to compare the histological features with those of the major and minor salivary glands.

Methods: Tubarial glands, major salivary glands (submandibular, sublingual, parotid), and minor salivary glands (buccal, labial, lingual) were excised from 12 cadavers (3 females, 9 males). Formalin-fixed, paraffin-embedded, hematoxylin and eosin-stained sections were assessed by light microscopy. Morphological features including lobule size and number of ducts were analyzed using ImageJ software. The quantitative data were compared statistically between tissue samples using a one-way ANOVA.

Results: Sixteen parotid glands, 17 submandibular glands, 14 sublingual glands, 8 buccal glands, 11 labial glands, 8 lingual glands, and 16 tubarial glands were excised from 11 cadavers. Tubarial gland sections showed clusters of mostly simple single-lobule minor submucosal glands composed of predominantly mucinous acini surrounded by loose connective tissue, with excretory ducts running parallel the mucosa. When combined, major salivary glands had significantly larger lobules ($p<0.0001$) and greater number of ducts than tubarial glands ($p<0.0001$) and minor salivary glands ($p=0.0282$). There was no significant difference between tubarial glands and minor salivary glands on the above parameters ($p=0.3258$ and $p=0.0966$, respectively).

Conclusions: The proposed tubarial glands are minor salivary glands by morphological criteria such as lobule size and number of ducts. The presence of minor seromucinous glands around the torus tubarius has been well known and the presence of major salivary glands in this area would be unexpected. Our study does not explain the increased uptake of PSMA ligand seen bilaterally in the nasopharynx in the Valstar et. al. study, but it does exclude the proposed explanation of previously unrecognized major salivary glands located in this site.

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EXTRAGLOMERULAR VASCULITIS AS AN INDEPENDENT PROGNOSTIC FACTOR IN ANCA-RELATED GLOMERULONEPHRITIS: A VALIDATION STUDY IN A CALGARY COHORT

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Introduction:
Anti-neutrophil cytoplasmic antibody (ANCA)-related glomerulonephritis (GN) is the most common etiology for a rapidly progressive glomerulonephritis (RPGN) worldwide. The histopathologic classification by Berden, the Renal Risk Score (RRS) and the Mayo Clinic Chronicity Score (MCCS) have all been developed to predict end-stage kidney disease (ESKD)-free survival in their cohorts. The effect of extraglomerular vasculitis (e.g. arteritis), however, has not been thoroughly validated. This study aims to investigate the impact of arteritis on the prognosis of ANCA-related GN patients in Calgary, with a primary outcome of ESKD-free survival. In addition, the Berden, RRS, and MCCS systems are validated within our cohort.

Methods:
Kidney biopsies scanned into the University of Calgary's renal biobank from 2016-2018 were screened to select those meeting criteria for a diagnosis of ANCA-related GN. Clinical and demographic information was retrieved from electronic medical records while histopathological scoring of cases was done by analyzing digital slides from the Biobank.

Results:
In total, 78 cases were included in our study, 16 of which showed the presence of arteritis (20.5%). Our cohort validated all three prognostic scoring systems (Berden, RRS, and MCCS). In addition, significantly worse outcomes were seen for patients with MPO positivity, compared to PR3. However, the results from our study did not reveal a significant difference in ESKD-free survival for ANCA GN cases with arteritis compared to those without arteritis.

Conclusion:
Our study reveals that MPO status is a worse prognostic indicator in patients with ANCA GN. In addition, all three prognostic systems (Berden, RRS, and MCCS) could be validated with our cohort. The findings in this study do not support the concept that ANCA-related GN with arteritis represents a different subtype of ANCA vasculitis with a worse prognosis. Additional validation with larger cohorts, including in the form of prospective studies, is needed to further investigate this relationship.

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A NOVEL SIMULATION METHOD FOR TEACHING GROSS DISSECTION SKILLS IN ANATOMICAL PATHOLOGY

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Competent dissection of surgical specimens is an essential component of training for future pathologists' assistants and anatomical pathologists. Errors during grossing can significantly impact pathologist's diagnostic interpretation and assessment of reporting parameters such as staging, primary tumor location and surgical margins that ultimately guide patient's diagnosis and future treatment. Simulation training is well established and utilized in other training programs as a useful teaching tool, especially for procedural skills. Our study looks at whether simulation can effectively be used to teach gross dissection of complex specimens in a low-risk environment within an anatomical pathology training program. The study aims are two-fold: first create a dissectible, three-dimensional model of a pancreaticoduodenectomy (Whipple's) specimen; and second, determine whether it improves learning outcomes and learner confidence. We recruited anatomical pathology residents, pathologists' assistant master's students and practicing pathologists' assistants from our institution to participate in a workshop for grossing pancreaticoduodenectomy specimens. During the workshop, participants dissected a simulated specimen according to our institution's standard operating procedure. A short-answer knowledge test was performed by participants before and after the workshop. Participants' confidence in specimen orientation and gross dissection following simulation was assessed using a Likert-scale questionnaire. Our preliminary results show that simulation improved participants' (n =27) knowledge scores between a pre- (64.7% (95% CI 5.3)) and a post-simulation test score average (87.3% (95% CI 2.3)). Similar trends were observed in the self-reported confidence in both orientation and grossing improved following the simulation workshop; reported pre-workshop specimen orientation confidence increased from 2.8 ± 1.0 SD to 4.3 ± 0.6 SD and pre-workshop gross dissection confidence score of 2.3 ± 1.0 SD increased to 3.5 ± 0.9 SD. Our results suggest that similar to other established fields, our novel simulation model is a valid teaching method that improves gross dissection knowledge and confidence in anatomical pathology trainees.

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LAMTOR4 is Associated with Lethal Prostate Cancer and Its Knockdown Decreases Cell Proliferation, Invasion, and Migration In Vitro

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Introduction:
Late endosomal/lysosomal adaptor, MAPK and mTOR or LAMTOR is a scaffold protein complex that senses nutrients and integrates growth factor signaling. LAMTOR4 is one of the main components of the regulator-complex. The role of LAMTOR4 in tumorigenesis still unknown. However, there is a considerable possibility that LAMTOR4 is directly involved in tumor cell proliferation and metastasis.

Methods:
In the current study, we investigated the protein expression of LAMTOR4 in a cohort of 314 men who were undergoes transurethral resection of prostate (TURP) consisting of incidental, advanced and castrate resistant cases. We also correlated the data with ERG and PTEN status and clinic- pathological features including, Gleason score, patients' outcome. Additionally, we performed in vitro experiments utilizing knockdown of LAMTOR4 in LnCAP prostate cell line and finally performed RNA expression assessment using TCGA prostate adenocarcinoma (TCGA-PRAD) to check the genes and pathways associated with *LAMTOR4* overexpression in PCa patients.

Results:
We found that, high LAMTOR4 protein expression was significantly associated with poor overall survival (OS) (HR: 1.44, CI: 1.01-2.05, p = 0.047) and unfavorable cause specific survival (CSS) (HR: 1.71, CI: 1.06-2.77, p = 0.028). Additionally, when high LAMTOR4 expression combined with PTEN-negative cases (score 0), we found significant poorer OS (HR: 2.22, CI: 1.37-3.59, p = 0.001) and CSS (HR: 3.46, CI: 1.86-6.46, p < 0.0001). Furthermore, ERG-positive cases with high LAMTOR4, exhibited lower OS (HR: 1.98, CI: 1.18-3.31, p = 0.01) and CSS (HR: 2.54, CI: 1.32-4.87, p = 0.005). In vitro assessment shown knockdown of LAMTOR4 decrease PCa cells proliferation, migration, and invasion. Our data further showing that knockdown of LAMTOR4 in LnCAP cell line significantly dysregulated mTOR pathway and tumorigenesis associated Pathways.

Conclusion:
Given its association with more aggressive forms of prostate cancer and its involvement in mTORC1 signaling, LAMTOR4 may considered a potential target for therapeutic intervention in PCa. Inhibiting components of the mTOR pathway, including LAMTOR4, might offer a strategy to inhibit tumor progression and metastasis in prostate cancer.

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PREVALENCE, TREATMENT, AND OUTCOME OF REAL-WORLD FUSION-POSITIVE NON-SMALL CELL LUNG CANCER (NSCLC) IN ALBERTA

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Introduction:
The clinical management and prognosis of NSCLC has been greatly impacted by the discovery of oncogenic driver genes and use of targeted therapies. Implementation of funded, standard-of-care next-generation sequencing (NGS) in Alberta has served to reduce barriers to the identification of oncogenic drivers of disease, but obstacles remain with respect to reimbursement restrictions on molecularly targeted therapies.[1,2] The real-world clinical management and outcome of fusion-positive NSCLC in Alberta is a current knowledge gap.

Methods:
Alberta patients with advanced/metastatic (M1) NSCLC, meeting the criteria for funded provincial genomic sequencing between April 2022 and May 2023, received reflexive (SNV-hotspot driver negative) RNA-NGS by Alberta Precision Laboratories. Testing was performed by Archer FusionPlex (IDT) with sequencing on Genexus (ThermoFisher). Demographic, clinical, treatment and outcome details were extracted from a University of Calgary based, province-wide, real-world outcomes registry of patients treated in routine practice.

Results:
Testing identified 47 individuals with an oncogenic fusion or isoform: METΔEX14 (38%), BRAF oncogenic-isoform (2%), and fusions: ALK (34%), RET (11%), ROS1 (9%) and FGFR2/3 (6%), identified/reported a median 1.1 months following histological diagnosis. Systemic anti-cancer therapy (SACT) was received by 27 (57%) of the cohort. At the time of SACT initiation, where funded, targeted options existed (ALK, ROS1), 100% of patients received targeted therapy; conversely, approved but unfunded therapies were (METΔEX14, RET), saw only 29% receive these treatments. Survival time post-M1NSCLC), while not reaching statistical significance, was seen to be higher in those receiving targeted, compared to non-targeted therapies (71.1 vs. 22.6 months, p=0.08).

Conclusion:

This study provides an overview of the number, variety, and clinical management of oncogenic fusions and isoforms found in NSCLC via NGS-testing in Alberta. Lack of provincial funding impacts the rate of approved targeted therapy uptake. This is meaningful, as the use of targeted therapies in this real-world cohort shows a

numerically longer survival time, which is clinically meaningful in the context of M1-NSCLC. Inclusion of NGS-based genomic profiling and funding of targeted therapies as part of standard of care within the public healthcare system supports appropriate clinical management and optimizes patient outcome within real-world Alberta NSCLC patients with actionable oncogenic fusions.

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WHERE IN THE WORLD IS GNAQ MUTATED MELANOMA: A QUALITY ASSURANCE PROJECT

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Introduction:

The “Melanoma Panel”, a mass array panel to identify common mutations in melanoma, has been in use in Calgary since 2018 and is applied in cases of locally advanced or metastatic melanoma. GNAQ and GNA11 are genes rarely mutated in cutaneous melanoma but are often found in uveal melanoma and melanoma arising from a blue nevus. However, these lesions are considerably less common than cutaneous melanoma. Uveal melanoma typically has little response to traditional systemic melanoma therapy, therefore establishing the eye as the primary site can significantly alter the course of clinical management. Here, we sought to interrogate the site of origin for GNAQ or GNA11 mutated metastatic melanomas within our local population.

Methods:

We performed a retrospective chart review on all GNAQ or GNA11 mutated tumors identified by the Calgary Melanoma Panel from its inception in 2018 to November 2023. Tumors were classified by tumor type and as either primary or metastatic. In the case of metastatic melanoma of originally unknown origin, the site of origin was determined based on the impression of the treating clinician.

Results:

There were 39 GNAQ/GNA11 mutation positive lesions with chart available for review. 17 were non-metastatic lesions and 22 were metastatic melanoma. 91% (20/22) of the metastatic lesions and 100% (16/16) of the metastatic liver lesions were of uveal origin. 9% (2/22) of the metastatic tumors did not have an identified primary site. One of the patients with uveal melanoma presented with metastasis and the site of origin was identified based on the molecular profile.

Conclusion:

In our cohort, 100% of metastatic melanoma in the liver and 91% of metastatic melanoma to any site with GNAQ or GNA11 mutations were of uveal origin. This result has prompted a change to the molecular pathology comment to suggest ophthalmic examination be considered in cases where a GNAQ or GNA11 mutation is identified in a patient with no known melanoma history.