Department of Pathology & Immunology
Baylor College of Medicine

Trainee Research Symposium

Thursday, April 17, 2025
8:00 am - 1:30 pm

Cullen Auditorium, Rayzor Lounge
1 Baylor Plaza, Houston, TX 77030

Zoom Link: https://bcm.zoom.us/j/96977768606?pwd=qq2ES0KPOZkmyrTO0HqcUc57p8GMBV.1
Password: 974434

Program

7:30 - 8:00 am	**Breakfast**
8:00 - 8:05 am	**Welcome and Opening Remarks** **Martin M. Matzuk, MD, PhD and** **Jun Teruya, MD, DSc**
8:05-8:55 am	**Faculty Presentations** **Moderator: Sridevi Devaraj, PhD, DABCC, FRSC** **Kalyani R. Patel, MD** *"Biliary Atresia: Novel Insights in Portal Vein Pathology"* **Ramakrishna Kommagani, PhD** *"Gut microbiota: The Yin and Yang of endometriosis"*
9:00-9:50 am	**Larry E. Kane, MD, Memorial Lecture** **Huda Y. Zoghbi, MD** *"Rett syndrome and MECP2 disorders from the clinic to the bench and back"*
9:50-10:10 am	**Q & A**
10:10-10:20 am	**Break**
10:20 -11:20 am	**Oral Trainee Presentations: Session 1** **Moderator: Angshumoy Roy, MBBS, PhD** **Sydney Parks, BA** **Mary Clay Bailey, MD** **Heather C. Binns, PhD** **Goutham Davuluri, PhD**
11:20 am -12:05 pm	**Oral Trainee Presentations: Session 2** **Moderator: William Decker, PhD** **Nalini Bisht, MS** **Georgia Huffman, MD** **Nazmin Bithi, PhD** **Larissa Nitschke, PhD**
12: 05 -1:05 pm	**Poster Session and Lunch** **Boxed lunch will be provided**
1:05 -1:30 pm	**Awards Session and Closing Remarks** **Martin M. Matzuk, MD, PhD** **James Versalovic, MD, PhD** **Andrea Marcogliese, MD**

Trainee Research Committee: Drs. Thomas A. Cooper, Choladda V. Curry, William Decker, Sridevi Devaraj, Ashley Holloman, Martin M. Matzuk, Angshumoy Roy, Jun Teruya, and Jing Wang, Chief Residents (Drs. Dodson and Huffman), Associate Chief Residents (Drs. Rachel Guest and Katelyn Moss), and Dr. Sonalben Italiya (PGY2).

Keynote Lecture

— Larry E. Kane, MD, Memorial Lecture —

"Rett Syndrome and MECP2 Disorders from the Clinic to the Bench and Back"

Huda Y. Zoghbi, MD

Investigator, Howard Hughes Medical Institute
Director, Jan and Dan Duncan
Neurological Research Institute
Research-in-Chief, Texas Children's Hospital
Distinguished Service Professor, Baylor College of Medicine

Faculty Presentations

— Clinical Faculty —

"Biliary Atresia: Novel Insights in Portal Vein Pathology"

Kalyani R. Patel, MD

Medical Director, Anatomic Pathology Informatics
Pediatric Pathology
Texas Children's Hospital
Associate Professor, Pathology & Immunology
Baylor College of Medicine

— Research Faculty —

"Gut Microbiota: The Yin and Yang of Endometriosis"

Ramakrishna Kommagani, PhD

Associate Professor, Pathology & Immunology
Baylor College of Medicine

Trainee Oral Presentation Abstracts

Trainee Poster Presentation Abstracts

Trainee Poster Presentation Abstracts

TGFBR2 Coordinates the Endometrial Response to Estrogen, Preventing Endometrial Hyperplasia and Associated Infertility

Sydney E. Parks,[1,2,3] Suni Tang,[1,3] Anna Catherine Unser,[1,3] Vanessa J. Joseph,[1,3] Ananya L. Bhonsley,[1,3] Eunbi M. Chung,[1,3] Dominique I. Cope,[1,3] and Diana Monsivais[1,2,3,4]

[1]Department of Pathology & Immunology, [2]Cancer and Cell Biology Program, [3]Center for Drug Discovery, [4]Dan L. Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX 77030, USA.

Introduction

Approximately 25% of pregnancies experience complications or result in pregnancy loss, and women seeking fertility care are 10x as likely to have endometrial hyperplasia. Thus, defining the signals that coordinate endometrial receptivity and maintain healthy pregnancies are critical for improving both pregnancy outcomes and women's overall health. To identify the mechanisms controlling the dialog between TGFβ/estrogen (E2)/progesterone (P4) signaling during early pregnancy, we generated a new mouse model with conditional inactivation of the TGFβ type 2 receptor, TGFBR2, using progesterone receptor cre (PRcre) ("*Tgfbr2* cKO").

Methods

We performed 6-month fertility trials to determine the fertility of *Tgfbr2* cKO females and timed mating analyses to assess key pregnancy processes at 4.5, 5.5, 6.5, 8.5, and 12.5 days post coitus (dpc). Key markers of these processes, such as decidualization, were measured via qPCR and by immunohistochemistry and immunofluorescence staining of FFPE implantation site sections. Further investigation into the E2/P4 response of *Tgfbr2* cKO females was conducted using mouse endometrial epithelial organoids.

Results

6-month fertility trials demonstrated that *Tgfbr2* cKO females were infertile (controls, 584 pups, n=6; *Tgfbr2* cKO, 1 pup, n=6), and had a significantly reduced overall survival compared to their control counterparts. *Tgfbr2* cKO females that survived the 6-month trial all had reproductive tract masses (6/6), with one also presenting with metastases to the lungs. Analysis of female virgins demonstrated that even by 14 weeks of age, *Tgfbr2* cKO females exhibit endometrial epithelial hyperplasia and a near total loss of glandular FOXA2 – a known tumor suppressor in the endometrium. Timed mating analyses demonstrated that defects in pregnancy occurred during the peri- and post- implantation stages, where compared to controls, *Tgfbr2* cKO mice displayed fewer implantation sites at 4.5 days post coitum (dpc), 5.5 dpc, 6.5 dpc, and 8.5 dpc. Almost no implantation sites were recovered at 12.5 dpc in the *Tgfbr2* cKO mice, indicating that embryo resorption was likely completed by this point. Molecular analyses of control and *Tgfbr2* cKO implantation sites indicate a dysregulation of early pregnancy starting at implantation, that worsens throughout pregnancy and results in abnormalities in decidualization, uterine natural killer cell (uNK) invasion, and angiogenesis. At 4.5 dpc, implantation sites of *Tgfbr2* cKO mice demonstrate an increase in E2-response genes such as *Lifr*, *Lif*, *Lcn2*, and *Muc1*, suggesting unopposed E2 response during early pregnancy. At 5.5 dpc, implantation sites of cKO females demonstrate a significant decrease in progesterone receptor (PR) expression. When treated with E2 and P4, endometrial epithelial organoids from *Tgfbr2* cKO mice displayed increased *Esr1* and E2-response genes, further suggesting a connection between SMAD4 and estrogen receptor (ER) in the mouse uterus. Analysis of published genome-wide ER and SMAD4 binding datasets revealed overlapping ER and SMAD4 binding peaks within the PR promoter.

Conclusion

We show that *Tgfbr2* is crucial for maintaining a healthy pregnancy by regulating key processes. We also propose that TGFBR2, through its downstream transcription factor, SMAD4, modulate PR expression by co-binding its promoter with ER, ultimately controlling epithelial hyperplasia and pregnancy processes such as implantation, decidualization, and uNK recruitment.

Studies were supported by Eunice Kennedy Shriver National Institute of Child Health and Human Development grants R00-HD096057, R01-HD105800. Sydney Parks receives support from NIH T32 Training Grant Diversity Supplement (3T32HD098068-05S1; PI: Dr. Stephanie Pangas). Diana Monsivais, Ph.D. holds a Next Gen Pregnancy Award (NGP10125) from the Burroughs Wellcome Fund.

Potential Therapeutic Targets

Mary Clay Bailey[1], Maryam Shafiekhani[1], Horatiu Voicu[1], Jennifer Scull[1,2], Julie Gastier-Foster[1,2], Frank Y. Lin[3], Murali Chintagumpala[3], Dolores Lopez-Terrada[1,2,3], Kevin Fisher[1,2], D. Will Parsons[3,4], Carrie Mohila[1,2], Angshumoy Roy[1,2,3]

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466/500 words

INTRODUCTION

The *FGFR1-4* genes encode four highly conserved fibroblast growth factor receptor tyrosine kinases (RTKs) involved in the MAP kinase pathway. Oncogenic *FGFR* gain-of-function mutations are recurrent alterations in central nervous system (CNS) tumors in children and adults and may provide potential therapeutic targets. Here we describe the spectrum of FGFR alterations, including rare structural variation (SV) type of alterations, in a series of CNS tumor patients at a pediatric hospital.

METHODS

An institutional clinical laboratory genomic database was searched from 2017-2024 for CNS tumors with oncogenic *FGFR1-4* alterations reported on targeted clinical genomic panels (TCH Solid Tumor Panel). Targeted genomic sequencing was performed on DNA and RNA derived from FFPE tissue using Roche KAPA hybrid capture and Archer FusionPlex systems with Illumina System. NextGENe v2.4.1.2 and Platypus v0.8.1 were used to call single nucleotide variants (SNV) and indels <25 bp; longer indels (>25 bp) were called by Pindel v0.2.5 and Delly v0.8.1. Copy number variant (CNV) analysis was performed using CNVkit v.0.9.3. Additional DNA methylation analysis was performed on select cases based on subtype diagnosis and specimen availability. Histology, initial diagnosis, and integrated diagnosis were reviewed.

RESULTS

Among 465 patients with CNS tumors tested during 2017-2024, a total of 29 patients (6.2%; 17M/12F; 28/29 of age 1-18 years)were identified to harbor oncogenic *FGFR* alteration-positive CNS tumors. All *FGFR*-positive tumors were classified as gliomas or glioneuronal tumors (low-grade tumors 23/29, 79.3%; high-grade 6/29, 20.7%). Low-grade tumors included polymorphous low-grade neuroepithelial tumor of the young (PLNTY, n=6), pilocytic astrocytoma (PA, n=6), dysembryoplastic neuroepithelial tumor (DNET, n=6), as well as low-grade glioma (LGG, n=4), and rosette-forming glioneuronal tumor (RGNT, n=1). All high-grade tumors were high-grade gliomas (HGG). Tumor *FGFR* alterations were mutually exclusive with respect to individual *FGFR* genes and comprised *FGFR1* (n=19, 65.5%), *FGFR2* (n=8, 27.5%), and *FGFR3* (n=2, 7%); no alterations were detected in *FGFR4*. Different alteration types were detected with structural variations (SV) more common (21/29, 72.4%) than SNV (8/29; 27.6%). *FGFR* SVs comprised most commonly gene fusions that involved all 3 (*FGFR1-3*) genes (n=11/21; 2 *FGFR1*, 7 *FGFR2*, and 2 *FGFR3* fusions) or tyrosine kinase domain partial gene duplications (TKD-D) (n=9/21) seen exclusively in the *FGFR1* gene. In contrast, all SNVs were limited only to the *FGFR1* gene. Oncogenic *FGFR* alterations demonstrated known associations with tumor

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types and genomic landscapes, frequently the sole alterations in low-grade tumors, but along with tumor-defining histone and other driver alterations in all HGG tumors.

DISCUSSION and CONCLUSION

This study reports on the prevalence and types of FGFR alterations seen in pediatric CNS tumors. *FGFR* SVs are more common in pediatric CNS tumors compared to SNVs, especially in low-grade tumors, highlighting the need for effective SV detection methods for fusions, CNV, and TKD-D alterations. *FGFR* alterations are well-known contributors to pediatric CNS tumor pathogenesis and have the potential to be targeted by tyrosine kinase inhibitors.

Trainee Oral Presentation Abstracts

Impact of Diagnostic Stewardship for Broad Range Polymerase Chain Reaction Testing in Pediatrics

Heather C. Binns[1,2], Coreen L. Johnson [1,2], James J. Dunn [1,2], Denver T. Niles [1,2]

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[2] Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX

Introduction: Broad range polymerase chain reaction with sequencing (BRPCR) is an advanced infectious disease diagnostic tool capable of identifying a myriad of bacterial, fungal and/or mycobacterial infectious etiologies directly from clinical specimens, often beyond the scope of conventional culture and molecular testing. However, the utility of BRPCR is limited by factors such as cost, turnaround time, and sensitivity. To ensure appropriate utilization of testing, Texas Children's Hospital implemented diagnostic stewardship review of all BRPCR orders in an effort to increase diagnostic yield and avoid unnecessary testing when an etiologic agent was already identified by conventional methods. This study evaluates the impact of diagnostic stewardship on BRPCR test utilization.

Methods: We conducted a retrospective chart review of 100 patients before and 100 patients after diagnostic stewardship implementation for BRPCR in October 2024. Total bacterial, fungal, and AFB BRPCRs sent post-stewardship and the associated cost savings were assessed. Positive BRPCR results were categorized as definitive (matching conventional test results), probable (pathogen identified only by BRPCR), possible (commensal organism potentially linked to disease), and unlikely (organism not associated with disease). For definitive BRPCR identifications, we determined whether they provided an earlier diagnosis than conventional methods.

Results: Post-stewardship, BRPCR test was performed for 88 of 238 requested orders among 100 patients, compared to 234 BRPCRs in the pre-stewardship cohort. This reduction led to an estimated cost savings of $69,818 in less than two months. A greater proportion of BRPCRs were performed on sterile specimens post-stewardship (7/36 vs 18/100, p= 0.04), and fewer polymicrobial BRPCR results were observed (2 vs 13, p= 0.22). The number of definitive bacterial BRPCR results decreased from 18 pre-stewardship to 3 post-stewardship (p= 0.17), with all definitive bacterial identifications occurring after conventional methods had already provided a diagnosis. The most common reason for stewardship-driven test cancellation was the prior identification of a causative pathogen by conventional methods. Probable and possible organism identifications were similar between the pre and post-stewardship (8/100 vs 3/36, p= 0.9). Overall, there was a higher rate of fungal organism identification post-stewardship (4/30 vs 2/75, p= 0.05). There were no positive mycobacterial BRPCR tests in either cohort.

Conclusion: Implementation of BRPCR diagnostic reduced unnecessary testing, resulting in significant cost-savings. The intervention prioritized testing of sterile specimens, leading to a reduction in polymicrobial results unlikely to affect clinical management. Additionally, stewardship minimized unnecessary testing when conventional methods had already identified a causative pathogen.

Splicing Factor SF3B1 Governs Innate Immunity to Coordinate Early Host Defense Against Pathogens

Goutham Davuluri[1], Thirumala-Devi Kanneganti[2] and Ramakrishna Kommagani[1,3&4.]

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Introduction: Pre-mRNA splicing plays a crucial role in shaping gene expression outcomes, yet little is known about how spliceosome influences innate immunity and the inflammatory responses to infection. Macrophages, the key innate immune cells, are central in detecting and responding to inflammatory cues including pathogens, organelle damage, or chemical cues. SF3B1 is a critical splicing factor in the U2 snRNP spliceosome complex, with mutations linked to myelodysplastic syndrome, and oncogenic transformation. However, the functional relevance and underlying mechanism of spliceosome action in host/macrophage responses to infection remain unexplored. In this study, we investigated the role of spliceosome function by modulating SF3B1 in macrophage function and host responses to infection.

Methods: We employed Bone Marrow Derived Macrophages (BMDMs) and a conditional knockout mouse model in which SF3B1 is ablated in the BMDMs using the Lysozyme-2 Cre driver. BMDMs were collected from 8-week mice and subjected to a series of macrophage-based studies and bacterial infections. In vivo studies were also carried out with septic shock studies on control and mutant mice.

Results: We found that SF3B1 expression is elevated at the protein level during monocyte-to-macrophage differentiation in murine bone marrow-derived monocytes and THP-1 cells. However, inhibiting SF3B1 expression didn't impact monocyte-to-macrophage differentiation, but significantly impaired their polarization to either M1-like or M2-like phenotypes. We also found that SF3B1 is indispensable for the inflammasome activation within macrophages in response to inflammatory stimuli. Moreover, we found that SF3B1 is vital for both the canonical and non-canonical inflammasome activation and the induction of pyroptosis owing to this inflammasome activation. Additionally, we showed that SF3B1 is essential for bacterial DNA and viral RNA-induced inflammasome activation in macrophages. Importantly, we demonstrated that loss of SF3B1 compromised NLRC4, NLRP3, and AIM2-induced inflammasomes in response to *S. typhi, S. aureus,* and *F. novicida* bacterial infection respectively. We also found a significant reduction in inflammatory cytokines expression (IL-1β and IL-18) and the concomitant reduction in pyroptotic cell death of macrophages with infection. Consistent with these findings, bacterial loads within macrophages are increased with loss of SF3B1 indicating a failure to clear the bacteria within the macrophages owing to impaired pyroptosis. Finally, mice with Sf3b1 ablation recovered from sepsis with reduced mortality and decreased immune cell infiltration in response to septic shock.

Conclusion: Overall, our findings highlight the critical role of SF3B1 in regulating inflammasome activation, cell death, and early host responses against bacterial infection. These insights open potential avenues for treating infectious diseases, autoimmune diseases, and diseases with chronic infections with splicing inhibitors.

Sexual Dimorphism in Dendritic Cell-Expressed CTLA-4 Regulation of Type 1 Immunity

Nalini Bisht[1,2,3], Sruthi Panja[4], Damilola Oyewole Said[1,2,3], Keenan Ernste[1,2,3], Vanaja Konduri[1,3], William Decker[1,2,3,5]

Baylor College of Medicine [1]Department of Pathology & Immunology, [2]Graduate School of Biomedical Sciences, [3]Dan L. Duncan Comprehensive Cancer Center, [4]Department of Biosciences, School of Natural Science, Rice University, [5]Center for Cell and Gene Therapy, Houston, TX 77030

Introduction: Cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) is an immune checkpoint molecule known as a critical regulator of T-cell immunity. While the expression of CTLA-4 has been extensively characterized in lymphoid cells, our prior published data demonstrate that myeloid lineage dendritic cells (DCs) express and secrete CTLA-4 in extracellular vesicles. We have reported that siRNA-mediated knockdown of CTLA-4 in DCs resulted in enhanced type 1 immunity in the form of $CD8^+$ T cell activation *in vitro* and enhanced antitumor responses *in vivo*.

Methods: To elucidate the specific immunomodulatory role of DC-CTLA-4, we developed and characterized a CD11c-Cre conditional knockout (cKO) mouse model of CTLA-4. The male and female CTLA-4 cKO and wildtype (WT) mice were subjected to steady state immunophenotyping by flow cytometry. Additionally, the cKO and WT mice were challenged with 200,000 B16-OVA melanoma tumor cells subcutaneously and the tumor burden and survival were evaluated.

Results: The cKO mice exhibit enhanced anti-tumor immunity compared to age-matched wildtype (WT) controls, with the female cKO mice exhibiting substantially stronger tumor inhibition in comparison to the male cKO mice. Additionally, at steady state, female cKO mice exhibited a strong type 1 immune response and reduced regulatory response, compared to age-matched WT controls. In contrast, this effect was not seen in steady-state male cKO mice. Interestingly, interrogation of DC populations in these mice showed elevated type 1 conventional DC (cDC1) populations in female cKO mice as compared to female wildtypes; however, elevated levels of cDC1 were not observed among male cKO mice.

Conclusion: The data demonstrate that DC-CTLA-4 is involved in a sexually dimorphic immunoregulatory process where female cKO mice exhibit a pro-inflammatory immune landscape and heightened anti-tumor immune activity across dendritic cells and T cells. Future studies will focus on the influence of sex hormones on this observed phenotype in CTLA-4 cKO mice.

Trainee Oral Presentation Abstracts

Analysis of Wastage, Savings, and Maternal and Pediatric Outcomes for Pooled Pathogen Reduced Cryoprecipitate versus Conventional Cryoprecipitate

Georgia Huffman, MD[1], Nathan Wilken, MD[1,2], JiaHoi Loh, MLS (ASCP)[2] , Maryam Fazal, MLS (ASCP) [2], In Lei, MS, MLS (ASCP) [2], Allison Myers, MSN, CCRN [2], Amir Navaei, MD[1,2], Lisa Hensch, MD[1,2], Derek Danner, MD[1,2]

[1]Department of Pathology, Baylor College of Medicine; [2] Department of Pathology, Texas Children's Hospital

Introduction

Hypofibrinogenemia is a significant cause of mortality in bleeding pediatric and obstetric patients. Cryoprecipitate is commonly used for replacement of fibrinogen in these patients. Conventional cryoprecipitate (CRYO, 5-unit pool) wastage is a common issue in hospitals due to its 6-hour shelf life. Pathogen-reduced cryoprecipitate (PRC, 4-unit pool), with a 5-day shelf-life, may reduce waste. The aim of this study was to evaluate wastage, safety, and efficacy in our pediatric and obstetric populations.

Methods

The blood bank LIS was reviewed to identify transfusion and wastage data for cryoprecipitate. Baseline wastage data was collected from January 2022 to September 2022. The study period, in which both CRYO and PRC were in use, was from October 2022 to December 2023. For CRYO wastage, all pools were reviewed to determine likelihood of usage if shelf-life had been 5 days. We determined pools were "highly likely" to have been used if >3 pools were issued in 5 days and "likely" to be used if 1-2 pools were issued in 5 days. We retrospectively reviewed the electronic medical record to collect age, weight, number of units, fibrinogen increment, and presence of transfusion reactions for pediatric (< 18 years) and obstetric patients receiving CRYO and PRC. Statistical analysis was performed using SPSS version 27 (IBM, NY).

Results

Baseline wastage was 13.3%. For the study period, total waste for CRYO and PRC was 10.2%. When separated, there was 13.0% wastage for CRYO and 3.0% wastage for PRC. The total cost of wasted units was $24,096 (CRYO: $19,096; PRC: $5,000). For CRYO pools, 40 were "highly likely" and 11 were "likely" to have been transfused with 5-day outdating similar to PRC, resulting in a possible savings of $13,640-$17,391 and yielding an adjusted total wastage 1.6-4.5%. The efficacy and safety data for pediatric and obstetric patients are listed in Table 1. There were no significant differences identified in patient demographics or fibrinogen response between the CRYO and PRC patients. One transfusion reaction occurred in the CRYO group.

Conclusions

Use of a dual inventory of cryoprecipitate did not result in reduced wastage as compared to institutional baseline. However, utilization of PRC is associated with decreased waste as compared to CRYO in our tertiary pediatric and obstetric hospital. No significant differences were identified regarding safety or efficacy in our pediatric or obstetric populations.

Validation of NGAL Assay in a Pediatric Population

Nazmin Bithi, Ridwan Ibrahim, Estella Tam, Radwa Almamoun, Ayse Akcan-Arikan and Sridevi Devaraj

Abstract

Background and Objectives: Acute kidney injury (AKI) is a significant clinical concern, especially in high-risk settings, due to its association with high morbidity and mortality rates. However, current diagnostic markers, such as serum creatinine, have limitations in detecting early-stage AKI, as they often fail to indicate renal impairment until substantial kidney damage has occurred. Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a promising biomarker, as it is rapidly upregulated following kidney ischemia, where it helps reduce toxicity and promotes tubular cell proliferation via heme oxygenase-1. This study aimed to validate the BioPorto ProNephro AKI™ turbidimetric immunoassay for urinary NGAL on the Ortho Vitros XT7600 analyzer and assess its clinical performance for early AKI detection.

Design and Methods: We evaluated the BioPorto ProNephro AKI™ assay following CLSI guidelines for comprehensive validation process, including assessments of precision, linearity, accuracy, limit of detection (LOD), specificity, and reference range verification. Method comparison was conducted using 20 urine samples, while reference range verification was performed with 57 urine samples to ensure the assay's reliability. Additionally, a clinical study was performed using samples from 20 AKI samples to further assess assay performance and reliability. A clinical validation study was also conducted using 21 pediatric AKI samples to assess the assay's diagnostic performance.

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Results: The BioPorto ProNephro AKI™ turbidimetric immunoassay demonstrated excellent precision, with intra-assay coefficients of variation (CV) ranging from 1.3% to 1.8% and inter-assay CV values between 1.8% and 2.7%. Linearity was observed for NGAL concentrations between 18 and 1140 ng/mL, with dilution studies extending the measurable range to 15,000 ng/mL. Method comparison revealed high accuracy, with a strong correlation (r = 0.9836) and a bias of 7.2%. Limit of sensitivity (LOS) and specificity evaluations, including interference studies, showed no significant impact from pH, hemolysis, bilirubin, protein, or common drugs. Reference range verification in a pediatric population highlighted the assay's clinical utility for early AKI detection. Clinical validation using 21 pediatric samples showed high sensitivity (76.19%) and specificity (100%) for detecting AKI. The assay accurately identified samples with elevated NGAL levels indicative of AKI and had a low false-positive rate.

Conclusions: The BioPorto ProNephro AKI™ turbidimetric immunoassay on the Ortho Vitros XT7600 analyzer exhibits excellent performance for detecting urinary NGAL in pediatric patients, with high sensitivity and specificity. The clinical validation results suggest that this assay can significantly enhance early AKI detection, facilitating timely clinical interventions and improving patient outcomes.

Understanding the Mechanisms Underlying the Neurological Manifestations of Myotonic Dystrophy Type 1

Larissa Nitschke[1] and Thomas A. Cooper[1]

[1]Department of Pathology & Immunology, Baylor College of Medicine

Background: Myotonic Dystrophy Type 1 (DM1) is a progressive multisystemic disorder affecting 1 in 8,500 individuals. While primarily known for its skeletal muscle dysfunction, approximately 80% of DM1 patients exhibit neurological manifestations including cognitive impairment, sleep disturbances, and neuropsychiatric symptoms. The severity of these neurological features correlates with disease onset, with congenital and childhood-onset patients showing more profound neurological involvement. DM1 results from a CTG repeat expansion in the 3' untranslated region of the *DMPK* gene. Transcription of the expanded allele produces CUG repeat-containing RNA that forms nuclear foci and sequesters RNA-binding proteins of the Muscleblind-Like (MBNL) family. This sequestration results in the functional depletion of MBNL proteins, leading to widespread dysregulation of alternative splicing. While MBNL sequestration and mis-splicing of key MBNL targets have been shown to be responsible for the DM1 skeletal muscle phenotypes, the molecular mechanisms underlying DM1 neurological manifestations remain poorly understood, hindering the development of targeted therapies.

Methods: Progress in understanding DM1 brain disease has been limited by the lack of animal models that recapitulate the disease. To address this gap, we developed a doxycycline-repressible transgenic mouse model (CUG960) expressing 960 interrupted CUG repeats throughout the brain.

Results: The CUG960 DM1 brain mouse model exhibits widespread expression of CUG repeat RNA in the brain and recapitulates key molecular features of DM1 including nuclear RNA foci formation, MBNL sequestration, and alternative splicing dysregulation. Notably, we observed altered splicing of *Mapt* exon 10, which is expected to affect the 3R/4R Tau isoform ratio, a finding with potential implications for the cognitive phenotypes observed in DM1. Phenotypically, CUG960 mice demonstrate reduced brain weight, hyperactivity, and deficits in learning and memory. Utilizing the doxycycline-repressible system, we show that toxic RNA expression can be temporally controlled, enabling investigation of both developmental and adult-onset disease mechanisms. Furthermore, we established that CUG repeat RNA expression can be turned off at different timepoints, facilitating rescue studies to determine disease reversibility and identify critical therapeutic time windows.

Conclusions: The CUG960 DM1 brain mouse model addresses a significant unmet need in the DM1 research field by providing a valuable tool for investigating the neurological manifestations in DM1. This model system, with its ability to precisely control the disease-causing RNA in a time-dependent manner, will facilitate research into the molecular mechanisms underlying neurological DM1 phenotypes, the age-dependent effects of DM1 brain disease, the reversibility of disease features, and the identification of critical windows for therapeutic intervention.

ID# 1

Pleomorphic Hyalinizing Angiectatic Tumor: Exploring Potential Targeted Therapies and the Limitations of Biopsy

Authors: Mokhtar H. Abdelhammed, M.D., Neda Zarrin-Khameh M.D., M.P.H.

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Introduction:

Pleomorphic hyalinizing angiectatic tumor (PHAT) is a rare, locally recurring, non-metastazing mesenchymal tumor, characterized by ectatic, fibrin-filled vessels surrounded by a cellular proliferation of pleomorphic cells with low mitotic figures The tumor presents as a long-standing painless subcutaneous mass in the lower extremities. Here we present a patient with a left calf mass that was diagnosed as PHAT.

Case Presentation:

A 52-year-old woman with limited medical access presented with a painful, enlarging mass in her left calf and a 20-pound weight loss over two months. Although initially it was observed in 2019, her lesion was not managed. Follow-up CT and MRI scans revealed growth from 8.6 to 11.3 cm, with increased edema and possible necrosis. PET/CT indicated increased uptake which raised concern for malignancy along with lesion's increased growth and necrotic radiological features.

Pathologic and Ancillary Findings:

Biopsy revealed spindle cells with pleomorphic nuclei, foci of hemorrhage, and hemosiderin deposits. Immunohistochemistry showed vimentin positivity, weak CD99, and focal desmin, but negativity for CD34 and S100. A diagnosis of low-grade, undifferentiated soft tissue sarcoma was rendered. The patient received neoadjuvant chemo- and radiotherapy with modest response, followed by radical resection. The resected specimen had a necrotic, well-circumscribed lesion with spindle and pleomorphic cells, hemosiderin deposits, and hyalinized vessels, with 60% necrosis due to treatment. All margins were negative. Next-generation sequencing using a 648-gene panel identified MAP2K1, NRG1, ALK, XPC, RAD50, ZFHX3, MKI67, ATP7B, ZFHX3 and BCR of unknown significance with allele fractions from 15.8% to 57.1%. Tumor mutational burden was stable at 4.7 mut/MB.

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Conclusions:

PHAT is a rare, complex neoplasm with distinctive histology. Given its similarities to other neoplasms, diagnosis should ideally be based on resected specimens. We identified gene mutations of uncertain significance, with high variant allele fractions potentially influencing tumor behavior, though sequencing challenges remain. Our case highlights the role of chemo- and radiotherapy in PHAT management, beneficial both as neoadjuvant and post-resection options. Understanding PHAT's histopathology, IHC, and genetics aids derm/soft tissue pathologists in accurate diagnosis and reduces unnecessary interventions, ensuring better patient outcomes.

ID# 2

Adrenal Lymphangioma

Introduction

Adrenal lymphangiomas (AL) are a very rare benign vascular lesion, with only 55 cases currently reported in the literature. These lesions are typically unilateral and remain asymptomatic throughout life. The diagnosis of AL is established after surgical resection and microscopic examination.

Case Presentation

Here we present the case of a 31-year-old woman with an incidental adrenal mass that was found during workup of hematuria. CT scan showed a 5.1 x 2.9 cm lobulated, hypodense cystic lesion with coarse calcification center in the right adrenal gland. The patient subsequently underwent right adrenalectomy.

Pathologic and Ancillary Findings

The adrenal was serially sectioned to show a 5 x 2.5 x 1.7 cm multi-locular cortical mass filled with gelatinous contents and moderate peripheral calcifications causing flattening of the medulla without gross invasion. Representative sections of the mass were submitted for histologic evaluation. Microscopically, the mass had a multicystic lesion lined by flat endothelial cells. Focal hemorrhage and calcification were noted. The lining endothelial cells were highlighted by factor VIII immunohistochemical stain.

Discussion

Adrenal cysts are rare lesions which can be classified as endothelial cysts, pseudocysts, epithelial cysts, or parasitic cysts. Adrenal lymphangioma would be categorized as an endothelial cyst. Grossly, these lesions are typically unilateral with smooth borders and pure cystic structure. Microscopically, ALs are multiloculated cystic lesions lined by endothelial cells and are often filled with proteinaceous material. Positive immunohistochemical markers that are useful in the diagnosis of this entity are Factor VIII, CD31, CD34, and D-240. D-240 is specific for lymphatic endothelial cells, unlike CD34 and CD31 which will stain both vascular and lymphatic vessel endothelial cells. These lesions are negative for cytokeratin, which confirms the lymphatic, rather than mesothelial, origin of these lining cells. Adrenalectomy is often the appropriate treatment of large symptomatic ALs, while small asymptomatic cysts may be monitored conservatively with radiological surveillance. Patient's with ALs typically have excellent long-term prognosis.

ID# 3

Evaluating the Diagnostic Utility of PRAME and CD34 Dual Staining in Fibrosarcomatous Dermatofibrosarcoma Protuberans

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Abstract

Introduction:

Preferentially expressed Antigen in Melanoma (PRAME) is a melanoma-associated antigen that has shown its utility in distinguishing melanoma from benign melanocytic nevi. However, it is reported to express in various other malignancies. Fibrosarcomatous dermatofibrosarcoma protuberans (FS-DFSP) is a high-grade variant of dermatofibrosarcoma protuberans (DFSP), characterized by increased cellularity, mitotic activity, and nuclear atypia, with a higher risk of metastasis. Distinguishing FS-DFSP from DFSP can be challenging, particularly in cases with ambiguous morphological features. Immunohistochemically, most cases of conventional DFSP show diffuse and strong cytoplasmic expression of CD34. Nonetheless, there may be diminished or absent CD34 expressions in FS-DFSP. We present a case of FS-DFSP demonstrating loss of CD34 and positive PRAME expression.

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Case Presentation:

A 49-year-old African American male with a remote medical history of a major motorcycle accident presented with a growing anterior chest and neck mass. The patient reported a 2 cm flat scar in the same area, which had remained unchanged since the accident. Over a 12-month period, the mass had rapidly increased in size. He presented to the clinic with bleeding from the mass. Imaging studies revealed a circumscribed lobulated mass measuring approximately 6.0 cm within the soft tissues of anterior neck/upper thorax.

Pathology:

The incisional biopsy revealed a monomorphic spindle cell neoplasm with a storiform pattern, monomorphic nuclei, eosinophilic cytoplasm, and few mitotic figures. Immunohistochemistry showed strong CD34 positivity and negativity for pancytokeratin, SOX10, Factor XIIIa, SMA, desmin, and ERG, consistent with conventional DFSP. The patient underwent wide local excision which exhibited similar histopathological findings along with areas of increased cellularity, a herringbone pattern, greater nuclear atypia, and high mitotic activity (up to 32/10 HPF) with a Ki-67 index of 25-30%. CD34 was diffusely positive in conventional DFSP but lost in highly cellular areas. PRAME was negative in conventional DFSP but diffusely positive in these areas.

Conclusion:

In the evaluation of melanocytic lesions, PRAME is often used in conjunction with other markers including SOX10, Ki-67, HMB45, p16 and molecular findings providing a more comprehensive assessment of such lesions. Similarly, while larger studies are required to validate the CD34-PRAME dual staining approach in FS-DFSP, we recommend dermatopathologist to incorporate PRAME alongside CD34 and histopathological features to enhance diagnostic accuracy for challenging FS-DFSP cases. PRAME-positive cases of FS-DFSP can also be considered as a potential therapeutic target with PRAME-specific immunotherapy.

ID# 4

Diffuse Uterine Leiomyomatosis: A Case Series Highlighting Diagnostic Challenges of a Rare Condition

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Introduction

Diffuse uterine leiomyomatosis (DUL) is a rare entity characterized by innumerable confluent, smooth muscle nodules that replace the entire myometrium. It is often misdiagnosed as multiple typical uterine leiomyomas especially prior to surgery.

Method

A search of the pathology database for DUL revealed four cases diagnosed on hysterectomy specimens between 2018 and 2024.

Results

Patients, aged between 39 to 42 years, presented with persistent pelvic pain and a markedly enlarged multifibroid uterus on pelvic examination and imaging. In all cases, gross examination revealed a symmetrically enlarged uterus with multiple subserosal, intramural, and submucosal nodules distributed throughout the myometrium. These nodules had a white-tan whorled cut surface, with some degenerative changes resembling leiomyomata. On microscopic examination, the myometrium was entirely replaced by a confluent, poorly circumscribed, multinodular proliferation of spindled smooth muscle cells without cytologic atypia or increased mitotic activity. There was a mild to moderate increase in cellularity with focal or diffuse perivascular cellular proliferation. While cleft-like spaces seen in leiomyomas were seen, no nodules within vascular spaces were noted. One patient had associated peritoneal leiomyomatosis. All patients had a benign post-operative course.

Conclusions

DUL is a rare histopathologic diagnosis made on hysterectomy specimens. Multifibroid uterus in the reproductive age group is the typical clinical presentation. Pathologic differential diagnoses include multiple leiomyomata, diffuse myometrial hypertrophy, intravascular leiomyomatosis and endometrial stromal sarcoma; correct identification of this rare benign entity and distinction from above mentioned differentials, especially the latter two that require additional clinical intervention, is of utmost importance.

ID# 5

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ID# 6

Myoepithelioma Masquerading as Lipoma: A Case Report

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Introduction:

Myoepithelioma is a rare soft tissue neoplasm composed exclusively or predominately of cells with myoepithelial phenotype. It often presents as a diagnostic challenge due to its significant architectural heterogeneity and immunohistochemical similarities to mesenchymal or neural/melanocytic tumors. We present this rare case of myoepithelioma, which was thought to be a lipoma, clinically.

Case presentation:

The patient is a 50-year-old male without significant past medical history who presented with a 5 mm subcutaneous nodule of the right upper abdomen. The nodule had been present for at least ten years and fluctuated in both size and tenderness. Ultrasound of the nodule revealed increased vascularity. The patient was then referred to general surgery for removal of the nodule.

Pathology:

Gross examination revealed a well-circumscribed, tan-yellow soft tissue mass with a smooth capsule. Histological examination of the nodule revealed a neoplasm composed of bland-appearing epithelioid cells with partially nested architecture. The nuclei were monomorphic, round to oval and without significant cytologic atypia or mitotic activity. Interspersed between the cells was myxohyaline stroma. Immunohistochemical stains performed showed neoplastic cells with coexpression of S-100 protein, pancytokeratin, and GFAP. SMA demonstrated patchy staining and Ki67 showed a low proliferative index. Synaptophysin, chromogranin, CDX2, and CK20 stains were negative. The morphology and immunophenotype were consistent with the diagnosis of myoepithelioma.

Conclusions:

Myoepitheliomas are rare but well characterized tumors that are known to arise in the salivary gland, skin and soft tissues. Rearrangement of EWSR1 gene (22q12) is seen in about half of the cases. Most cases of myoepithelioma are benign, with recurrence occurring in up to 18%, particularly when the resection is incomplete. Malignant transformation into myoepithelial

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carcinoma is seen in approximately 5% of cases. Myoepithelial neoplasms can grossly mimic other common benign etiologies such as lipoma, as described in the present case. Therefore, histopathological evaluation is crucial for the diagnosis of lesions or masses that appear clinically benign, to assess the risk of recurrence or malignant transformation and ensure complete resection.

ID# 7

Lethal Case of Suspected DICER Syndrome in Neonate

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Introduction
DICER syndrome is a rare cancer predisposition syndrome involving mutations in DICER1 gene which codes for an RNase involved in siRNA/microRNA development. Altered RNA fragments produce a variable constellation of dysmorphia with benign and malignant tumors including congenital pulmonary airway malformation (CPAM), cystic nephroma, pleuropulmonary blastoma (PPB), and embryonal tumor with multilayered rosettes (EMTR). While the exact prevalence of DICER1 syndrome is unknown and complicated by incomplete penetrance, carriers of the pathogenic DICER1 variants have a markedly increased risk of neoplastic development when compared to the general American population.
Case Presentation
We present an 8-day-old premature female neonate with dysmorphic features, prenatally diagnosed lung cysts, and kidney abnormalities who despite aggressive medical treatment (including extracorporeal membrane oxygenation - ECMO), died from pulmonary complications and multiorgan failure. Postmortem and microscopic examination revealed a constellation of findings strongly implicating DICER1 syndrome including EMTR and lung cysts consistent with CPAM type 4 versus PPB type 1.
Pathology
Post-mortem examination revealed nasal bridge flattening, irregularly spaced digits, pectus excavatum with widely-spaced, inverted nipples, bilateral enlarged cystic lungs, renomegaly, uterus didelphys with double-outlet vagina, and a suprachiasmatic brain tumor. Microscopy identified cystic changes throughout all lung lobes consistent with CPAM type 4 versus PPB type 1 spectrum with focal desmin positivity. Neuropathology demonstrated embryonal tumor with multilayered rosettes (ETMR), CNS WHO Grade 4. While the majority of ETMRs demonstrate microRNA cluster alterations on chromosome 19q, a subset associated with DICER syndrome instead have *DICER1* mutation. Other pertinent histology included disordered folliculogenesis and immature renal development with discontinuous nephrogenic zone.

Conclusions
While genetic/molecular studies were declined, the calculated probability of independently developing a CPAM/PPB spectrum lung lesion and ETMR is 1 in 24.5 billion. Given the association with PPB, ETMR and genitourinary abnormalities, we favored the unifying diagnosis of DICER syndrome and recommended parental genetic testing in the autopsy report. Genetic testing may be declined or unavailable in limited-resource settings such as medical examiner offices. This case highlights the broad diagnostic capabilities of autopsy examination, the importance of recognizing syndromic associations, and the ever-evolving presentations of DICER1 syndrome.

ID# 9

Title: Analytical and Clinical Validation of the Quidel Microvue sc5B-9 Enzyme Immunoassay in a Pediatric Population

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Introduction:
The complement system plays a key role of surveillance for the immune system and its dysregulation has been implicated in different diseases. Soluble C5b-9 (sC5b-9) is a soluble form of the Terminal Complement Complex (TCC) that is released into the circulation with elevated levels associated with increased risk in patients with atypical uremic syndrome (aHUS) and other chronic inflammatory conditions. With the advent of eculizumab, a complement c5 inhibitor, proper testing for diagnoses and therapeutic monitoring are warranted. Also, sC5b-9 levels could increase during extracorporeal membrane oxygenation (ECMO) and serve as an early indicator of thrombotic microangiopathy (TMA).

Methods:
We evaluated both the analytical and clinical performance of the Quidel Microvue sC5b-9 Plus enzyme immunoassay. Analytical performance was evaluated with precision (intra- and inter-assay), linearity, interference studies and correlation with a reference laboratory. Reference intervals was established using plasma samples from control donors. Clinical performance of the assay was assessed using EDTA-anticoagulated plasma of patients who are on extracorporeal membrane oxygenation (ECMO) who have acquired von-Willebrand disease and patients with reduced ADAMTS13 activity.

Results:
The assay showed acceptable intra and inter-precision with a CV of less than 16% for both low and high levels. Linearity ranged from 12.6 to 160.66 ng/ml, while accuracy and method correlation studies with a reference laboratory yielded a correlation coefficient (R) of 0.96. Dilution studies revealed a clinical reportable range extending to 2560 ng/mL. Hemolysis, icterus and lipemia did not affect the assay at 500 mg/dl, 40 mg/dl and 1000 mg/dl respectively. Reference range in control donors was established at = 268.0 ng/mL (range from 128.0 to 268.0 ng/mL). Clinical performance of the assay in patients with low ADAMTS13 activity and acquired von-Willebrand disease (AVWS) revealed elevated sC5b-9 levels of 2241.5 ± 983 ng/mL and 1575.5 ± 756 ng/mL respectively suggesting complement activation in these patient cohorts compared with controls with sC5b-9 levels of 196.3 ± 37.4 ng/mL.

Conclusion:
The Quidel Microvue sC5b-9 plus EIA assay demonstrated acceptable analytical performance and clinical utility for monitoring complement activation in patients. Further studies will be done to correlate sC5b-9 levels with existing markers of complement activation.

ID# 10

An Intra-thoracic SMARCB1 (INI1) deficient neoplasm; A rare entity

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Background:

INI1-deficient carcinomas are a rare and aggressive group of tumors characterized by the loss of expression of the INI1 protein, which is encoded by the SMARCB1 gene. INI1, a core component of the SWI/SNF chromatin remodeling complex, plays a crucial role in maintaining normal cellular differentiation, proliferation, and apoptosis. Its loss has been associated with the development of various malignancies, including atypical teratoid/rhabdoid tumors (AT/RT), renal carcinomas, and certain soft tissue and epithelial tumors. While INI1-deficient carcinomas are more commonly observed in other organs, such as the kidney and central nervous system, the occurrence of this phenomenon in the lungs is exceptional.

Case Presentation:

We present the case of a 22-year-old woman with no significant past medical history, who presented to the emergency room with shortness of breath and was found to have a supraclavicular mass. Imaging revealed a right middle lobe pulmonary embolism and an 11 cm mass at the right lung apex, along with metastatic lymphadenopathy, direct invasion of the tumor to ribs, and superior vena cava compression. Further workup identified extensive bone metastasis. Eventually, the patient was admitted to the intensive care unit with increased respiratory distress and superior vena cava syndrome and eventually passed away within two months of the diagnosis.

Pathology Description:

A biopsy of the lung mass showed a poorly differentiated neoplasm with varied architectural patterns, including solid sheets, focal papillary formations, and isolated cells. Tumor cells exhibited epithelioid features, with occasional cells demonstrating eosinophilic cytoplasm, vesicular eccentric nuclei, and prominent nucleoli. Mitotic activity, including atypical figures, was observed. Immunohistochemical staining of the tumor showed diffuse positivity for Pan cytokeratin, CAM5.2, and EMA, along with focal positivity for cytokeratin 7, Napsin, and GATA3. Tumor cells were not reactive with cytokeratin 20, CD30, P40, SALL4, synaptophysin and TTF1. SMARCA4 (BRG1) expression was preserved, while immunostaining for SMARCB1 (INI1) showed diffuse loss of nuclear expression.

Discussion:

Despite its rarity, INI1-deficient lung carcinoma is associated with a poor prognosis due to its aggressive nature and limited response to conventional chemotherapy. This underscores the need for further research to better understand the molecular mechanisms underlying this rare carcinoma and to develop targeted therapies that could improve patient outcomes.

ID# 11

Enhanced Precision in Pancreatobiliary Cancer Staging: The Impact of Whole-Mount Axial Sectioning on Whipple Specimen Evaluation

All Authors:

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Background:

The Whipple procedure is a complex surgical treatment for pancreatic ductal adenocarcinoma (PDAC), ampullary carcinoma (AC), and distal common bile duct cholangiocarcinoma (CC). Meticulous gross examination of the specimen is crucial in determining the tumor epicenter and use the relevant staging protocol. Surveys from the Pancreatobiliary Pathology Society highlight significant disagreement regarding the definition of margins in this specimen, though involvement of pancreatic radial surfaces should constitute R1. This study aims to evaluate the utility of whole-mount axial sectioning to enhance the accuracy of diagnosis, staging, and determining other pathologic prognosticators in Whipple specimens.

Design:

In this method, we differentially ink the surgical margins (uncinate, pancreatic neck), radial surfaces (vascular groove, anterior serosal, and posterior fascial), and the lumen of the main pancreatic duct and common bile duct through probing. Following adequate fixation, the specimen is serially sectioned in the axial plane at 4-5 mm thickness, and each slice is

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submitted as a whole mount in a large cassette (Image 1). Pathologic evaluation of tumor epicenter, size, depth of invasion, margin status, and number of dissected lymph nodes are performed with gross-microscopic correlation.

Results:

We evaluated a total of 12 cases: 6 PDACs, 1 AC, 1 CC, and 4 intraductal papillary mucinous neoplasm (IPMN) without invasion (Table 1). Tumor epicenters were identified with a high degree of certainty. Accurate measurements of tumor dimensions in PDAC and depth of invasion in CC and AC could be performed microscopically from the whole slice, enabling precise T staging (Image 2). All cases had negative margins based on CAP guideline; however, 3 cases (2 PDACs, 1 CC) had vascular groove involvement, and 1 PDAC involved the anterior surface. The average lymph node yield was 21 (range 13-41). Evaluation of IPMNs with determination of main vs. branched ducts was easily achieved in all 4 cases.

Conclusion:

Whole mount axial sectioning is a very useful technique for pathologic evaluation of Whipple specimens. This method eases the assessment of tumor epicenter, tumor size, and other pathologic parameters due to improved preservation of anatomical structures and easier gross-microscopic correlation. Axial sectioning with whole mounting can be used as a standardized grossing protocol in institutions that already utilize whole mounting for other organs, like prostate.

ID# 12

Hydrophilic Polymer Emboli: An Incidental Finding on an Arteriovenous Malformation

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Introduction: Intravascular medical devices are commonly coated with hydrophilic polymers that can embolize and cause a variety of clinical outcomes ranging from asymptomatic to end-organ ischemia. Polymer coating emboli can rarely obstruct large vessels, leading to clinical and/or radiologic evidence of ischemia in defined vascular territories. Fragmentation of the polymer may also lead to occlusion of smaller vessels, where histological analysis is essential for identification, as these events are below the detection threshold for most imaging techniques.

Case presentation: A 36-year-old woman presented to the emergency room with sharp neck pain and difficulty moving her head for the past week. Cerebral angiogram showed a right anterior temporal lobe arteriovenous malformation. Partial embolization using Onyx 18 under fluoroscopic guidance and a later excision of the lesion was performed.

Pathologic and ancillary findings: Histological examination showed aggregates of thick and thin-walled vessels with intervening reactive brain parenchyma, consistent with arteriovenous malformation. A single vessel shows intraluminal basophilic, non-refractile and non-polarizable amorphous material, morphologically resembling polymer coating material.

Conclusion: Although extremely uncommon, the risk of embolization from polymer coating materials entering the bloodstream may be rising with the growing use of percutaneous intravascular diagnostic and therapeutic devices. Given how rare it is, polymer coating embolism is often difficult to diagnose in clinical practice, highlighting the need for a strong collaboration between clinicians and pathologists to document findings related to polymer embolism.

Histologically, polymer coating emboli have been described as intravascular aggregates of a predominantly basophilic, granular material that is non-refractile and non-polarizable. It may also appear as eosinophilic, gray-black, or colorless, and show amorphous, serpiginous, or lamellated structures. Associated foreign body reactions with giant cells, fibrinoid necrosis, neutrophilic response, and intravascular degradation can also be seen.

In our patient, the presence of hydrophilic polymer coating embolic material is likely incidental without clinical consequences. However, in other clinical settings, it is important to recognize and report the presence of polymer coating emboli as these complications are often underrecognized as iatrogenic causes of morbidity and mortality.

ID# 13

Extraskeletal Myxoid Chondrosarcoma Mimicking Benign Lesions: A Case Study with Molecular Diagnostics

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Introduction: Extraskeletal myxoid chondrosarcoma (EMC) is a rare soft tissue sarcoma, primarily arising in the deep tissues of the proximal lower extremities. Clinically, it follows an indolent course but can exhibit more aggressive behavior, including local recurrence and metastasis. Histologically, EMC is characterized by an uncertain differentiation, abundant myxoid stroma, multinodular architecture, and uniform tumor cells arranged in cords, clusters, or networks. Accurate diagnosis is essential for optimal management.

Case Presentation: This is the case of a middle-aged woman who presented with six months of progressive left upper leg and gluteal pain accompanied by a growing mass. MRI of the left femur revealed a 23 x 10 x 7 cm mass involving the vastus lateralis and gluteus medius, radiologically consistent with an intramuscular myxoma. A biopsy was performed for further evaluation.

Pathology: Microscopically, the tumor exhibited a multinodular pattern with fibrous septa dividing pools of chondromyxoid material containing uniform spindle-shaped cells with eosinophilic cytoplasm arranged in cords. Areas of intratumoral hemorrhage with pigment deposition were observed. Immunohistochemistry revealed focal positivity for SMA and CD99, with a Ki-67 proliferation index of 10%. The tumor was negative for p53, pancytokeratin, HMB45, ALK, BCL2, desmin, CD34, and S100, effectively ruling out other diagnoses, including chondrosarcoma, melanoma, and myxoid liposarcoma. Further analysis with fluorescence in situ hybridization (FISH) for NR4A3 and DDIT3 showed rearrangements involving the NR4A3 gene region only, excluding myxoid liposarcoma and providing a definitive molecular diagnosis of EMC.

Conclusion: This case underscores the critical role of histologic, immunohistochemical, and molecular analyses in diagnosing EMC, particularly when radiologic findings mimic other benign lesions such as intramuscular myxoma. Misdiagnosis could significantly impact patient care and follow-up, as EMC requires distinct management strategies due to its potential for local recurrence and metastasis. This case highlights the diagnostic complexity of EMC and emphasizes the importance of a multidisciplinary approach to accurately identify and manage this rare entity.

ID# 14

Clinicopathologic and Cytogenetic Features of IgA Plasma Cell Myeloma: A Retrospective Review of 2024 Cases

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Introduction: IgA plasma cell myeloma (PCM)/multiple myeloma (MM) is less frequent than IgG PCM/MM and often exhibits a worse prognosis. Only a few studies focus specifically on the presentation and progression of IgA neoplasms, despite their prevalence and evolving diagnostic approaches. In this study, we retrospectively reviewed the multiple myeloma cases diagnosed at our institution in 2024, and focus on the clinicopathologic characteristics of IgA PCM/MM.

Methods: 18 cases of PCM/MM were identified. The medical record, pathology, flow cytometry, FISH, and cytogenetics reports were reviewed.

Results: Out of the 18 cases, 12 were IgG MM, 4 were IgA MM, 1 was IgD MM, and 1 was free light-chain MM. The average age at diagnosis was 62 years for IgA MM and 60 years for IgG MM. Compared to IgG MM, IgA MM showed higher bone marrow plasma cell involvement (50–95%, average 81%, versus 56.5 % in IgG MM). Additionally, all IgA MM cases exhibited poor prognostic markers on FISH/cytogenetic analysis, including t(4;14) (50%), 1q gains (75%), and MAF deletions (25%). In contrast, among the IgG MM cases, only 1 had t(4;14) (9%), and 5 had 1q gains (40%).

Conclusions: This small case series suggests that t(4;14) is more commonly associated with IgA myeloma, potentially contributing to its relatively worse prognosis and offering possible insights into its pathogenesis. Additionally, it advances the ongoing effort to study IgA PCM/MM as a distinct entity, highlighting its heterogeneous clinicopathologic and cytogenetic features. Further assessment with larger case volumes, tumor mutation profiling via next-generation sequencing, and treatment response correlations will be reviewed and studied.

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Case #	IG	Age	Sex	% of plasma cells in bone marrow	CD56	FISH
1	Free K	62	M	100%	Weak	1q gain, t(14:16)
2	IgA K	74	F	90%	Positive	t(4:14), hyperdyploidy
3	IgA K	75	F	95%	negative	1q gain, CCND-IGH
4	IgA K	56	F	50%	Positive	1q gain, trisomy 11, monosomy 13, hyperdiploidy
5	IgA L	42	M	90%	Positive	hypodyploidy, t(4:14)
6	IgD L	47	M	Unknown	negative	gain 1q, complex
7	IgG K	78	M	15%	Unknown	Insufficienct
8	IgG K	63	F	80	Negative	Variant CCND1::IGH fusion and 13Q deletion
9	IgG K	38	M	70-80	Positive	extra 7,9,11,15
10	IgG K	61	M	80	Positive	1q gain, complex
11	IgG K	54	M	70-80	Positive	gain 9,11,15
12	IgG K	42	M	95	Positive	1q gain, t(4:14), monosomy 13
13	IgG K	69	F	20%	Positive	1q gain, trisomy 11, monosomy 13, hyperdiploidy
14	IgG K	47	M	80%	Negative	tetrasomy 11
15	IgG L	72	F	20-25%	Unknown	Normal
16	IgG L	54	F	70-80	Positive	1q gain, extracopies of 7,9,11,15
17	IgG L	76	M	30%	Positive	1q gain
18	IgG L	73	M	30%	Negative	t(11:14)

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ID# 15

Performance of the Vitek® 2 AST-N807 and AST-XN30 Panels for Antimicrobial Susceptibility Testing of Gram-Negative Bacteria

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Introduction: The bioMérieux VITEK® 2 Gram-negative Susceptibility Cards (AST-N807 and AST-XN30) are automated microdilution antimicrobial susceptibility tests (AST) for evaluating susceptibility of aerobic Gram-negative bacilli for up to 32 antibiotics. This study aimed to validate the performance of these cards against standard of care (SOC) methods before clinical implementation.

Methods: Accuracy testing included 76 Gram-negative isolates, 43 of which were recovered from patient cultures and 33 which were obtained from the CDC Antimicrobial Resistance [AR] Isolates Bank. The organisms were representative of the common genera and species of gram-negative bacilli recovered from routine patient testing. Results from VITEK® 2 panels were compared to SOC methods which included VITEK AST-GN81, Pheno Accelerate, Kirby-Bauer disk diffusion, and E-test gradient diffusion.

Results: Initial overall categorical agreement (CA) was 92.3% (CDC AR: 93.1%, clinical: 91.4%) and overall essential agreement (EA) was 96.8% (CDC AR: 97.1%, clinical: 96.5%). Discrepancy analysis revealed that 13/16 initial very major errors (VME) and 12/15 initial major errors (ME) resolved after retesting or testing by another SOC method. Discrepancies were attributed to loss of resistance marker(s) during freeze-thaw processes. The final VME rate was 0.6% (3/536), with aztreonam at 5.9% (1/17), imipenem/relebactam at 11.1% (1/9), and ceftazidime/avibactam at 10.0% (1/10). Overall ME rate was 0.7% (3/430), with cefepime at 5.6% (2/36) and ciprofloxacin at 2.8% (1/36). The overall minor error (mE) rate was 7.0% (71/1017), with higher mE rates observed with ceftazidime (18.3%: 13/71), tigecycline (19.0%: 4/21), and nitrofurantoin (30.0%: 3/10). Precision testing, conducted over three days with triplicate testing of six quality control ATCC strains showed 100% CA and EA.

Conclusion: The VITEK® 2 AST-N807 and AST-XN30 susceptibility cards demonstrated acceptable performance for most antibiotics tested, meeting FDA criteria for CA, EA, and error rates. This validation supports the use of these cards for routine clinical use, with considerations for specific antibiotic limitations.

ID# 16

Oncocytic Lipoadenoma of the Parotid Gland: A Rare Case Mimicking a Malignant Neoplasm

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Introduction:
Oncocytic lipoadenoma (OL) is an uncommon salivary gland tumor, defined by the presence of oncocytic cells mixed with varying amounts of mature adipose tissue. So far, only 34 cases of OL have been documented, including the present case. The majority of these tumors have been reported in the parotid gland, with only a few cases involving the submandibular gland.

Case Presentation:
Our patient was a 72-year-old male who presented with a 6-month history of a right parotid mass. A computed tomography scan showed a mildly heterogeneous 3 cm mass in the right parotid gland, replacing most of the gland. The boundary between the mass and the native parotid tissue was indistinct. A superficial parotidectomy was performed.

Pathology:
Grossly, the cut surface of the right parotid gland revealed a well-circumscribed, tan-yellow, lobular, and rubbery mass close to the outer surface, measuring 3.8 x 3.8 x 3.3 cm. Microscopically, the mass was a well-demarcated, encapsulated neoplasm predominantly composed of oncocytes and adipocytes. The tumor also contained ductal elements with sebaceous and squamous metaplasia, scattered multinucleated cells, and chronic inflammation. Focal cellular atypia and rare mitoses were observed in ducts, likely representing reactive changes. A diagnosis of oncocytic lipoadenoma was rendered.

Conclusions:
Oncocytic lipoadenoma of the salivary gland remains poorly understood and is often overlooked in clinical diagnosis due to its rarity. Immunohistochemical stains are usually not necessary for diagnosis. Oxidative stress may play a role in the development of OL. Rearrangements of the high-mobility group AT-hook 2 (HMGA2) gene, seen in neoplasms such as lipomas and some pleomorphic adenomas, may also contribute to OL development. OLs have a benign clinical course and are typically treated with total or superficial parotidectomy, with no reported risk of recurrence.

Our report illustrates the characteristic histomorphology of oncocytic lipoadenoma of the parotid gland. It is crucial to recognize this rare entity composed of oncocytes, ducts, and adipocytes, and not to misinterpret the presence of oncocytes in adipose tissue as invasion. Additionally, it is important to be aware that ductal elements with sebaceous and squamous metaplasia may show cellular atypia and mitosis, mimicking a malignant process, as seen in this case.

ID# 17

Malignant Pleural Effusion of Renal Cell Carcinoma With Rhabdoid Features: A Rare Case Report

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Introduction: Renal cell carcinoma (RCC) comprises 85% of all primary renal neoplasms. Its presentation may be occult, and approximately 50% of cases are incidentally found upon imaging as in our case. Pleural metastasis is rare; only 1-2% of malignant pleural effusions are secondary to RCC. A rare and highly aggressive variant of renal cell carcinoma with rhabdoid features has been recently recognized.

Case presentation: A 55-year-old man came to the hospital with worsening shortness of breath secondary to recurrent large volume of pleural effusion. Thoracentesis showed an exudative pleural effusion with abundant malignant cells. Imaging studies also revealed a 5.3 cm right renal mass with lung and pleural nodules. Transbronchial biopsy from the lung and fine needle aspiration biopsy from the pleural nodule were performed.

Pathologic and ancillary finding: The biopsies from the lung and pleura showed high-grade malignant neoplasm with an immunohistochemistry profile (PAX8, CK 7, and CD 10 positive) favoring an adenocarcinoma of renal origin. Pleural and peritoneal fluid cytology revealed numerous isolated malignant cells with rhabdoid features. Rhabdoid features have been linked to higher grades and stages of renal cell carcinoma.

Discussion and Conclusion: Due to the highly vascular nature of the tumor, distant site metastasis is common in RCC but the presentation of RCC-complicated with malignant pleural effusion is rare. This case demonstrates that RCC can cause recurrent large-volume malignant pleural effusion, which has not been widely reported in the published literature. Only about 3.2–7.4% of RCC has rhabdoid features. Renal cell carcinoma with rhabdoid features is considered grade 4 by the International Society of Urological Pathology (ISUP) and recommended to report due to higher metastasis rates. It is a very aggressive tumor and is associated with a higher mortality rate and poor prognosis. In our case, the patient passed away shortly after the diagnosis of malignant pleural effusion.

ID# 18

Beware: Groove Pancreatitis May Be Concealing Adenocarcinoma

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Introduction: Groove pancreatitis (GP) is a unique form of chronic pancreatitis affecting the pancreatoduodenal groove, a potential space between the head of the pancreas, duodenum, and common bile duct (CBD). On imaging, it may present as either mass-like lesions or cystic lesions in the pancreatic groove, mimicking malignancy. Discerning between GP and pancreatic cancer is often challenging but clinically very important.

Case Presentation: A 57-year-old female presented with nausea, vomiting, and upper abdominal pain. Clinical history includes heavy long-term smoking, cholecystitis with cholelithiasis, and repeated attacks of pancreatitis in the prior year. An abdominal CT scan revealed a 4 cm duodenal mass continuous with a hypodensity within the pancreatic head. EUS imaging showed a thickened, mass-like duodenal wall with stenosis; endoscopy showed granular, erythematous duodenal mucosa, and normal ampulla. The pancreas showed calcifications, cysts, and hypoechoic strands consistent with chronic pancreatitis; pancreatic and common bile ducts were normal.

Pathologic and Ancillary findings: Initial biopsy of the duodenal mass revealed peptic duodenitis, and Brunner gland hyperplasia, but no dysplasia or malignancy. Despite a negative biopsy, based on imaging highly suggestive of malignancy and worsening symptoms, the patient underwent a Whipple procedure. Histology showed marked fibrosis of the pancreaticoduodenal groove, dilated and ruptured ducts containing proteinaceous material, acute and chronic inflammation, giant cell reaction, and Brunner gland hyperplasia, consistent with GP. An incidental 1mm focus of invasive adenocarcinoma was identified arising in an ampullary adenoma with low and high-grade dysplasia (pT1aN0). All resection margins and lymph nodes were negative for carcinoma.

Discussion and Conclusion: GP is rare and often misdiagnosed clinically as cancer arising either in the duodenum, distal CBD, or pancreas. Infrequently, adenocarcinoma may be present in the duodenal groove, making differential diagnosis with GP difficult. GP may be treated conservatively, but surgery is indicated when malignancy is suspected.GP is more frequent in males and is associated with alcohol abuse, smoking, and anatomical or functional obstruction of the minor papilla. Adenocarcinoma is very rarely associated with GP, especially in females, like in our case. Identifying typical features of GP in a Whipple specimen should not preclude the pathologist from a thorough examination for adenocarcinoma.

ID# 19

Clinical Impact of Factor XIII Concentrate Administration on Hemorrhagic Complications in Pediatric Patients with Acquired Factor XIII Deficiency during Extracorporeal Membrane Oxygenation Support

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Introduction: Bleeding is a major complication in patients on extracorporeal membrane oxygenation (ECMO). Factor XIII (FXIII) deficiency develops during ECMO. However, little is known about the FXIII therapeutic threshold level in ECMO settings and the effects of FXIII concentrate on bleeding management. Our study aimed to define the FXIII therapeutic threshold level and evaluate the FXIII concentrate effect in ECMO.

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Methods: Retrospective chart review was performed in pediatric ECMO patients with bleeding symptoms and FXIII deficiency who were treated with FXIII concentrate. The ISTH bleeding definition was used to classify hemorrhages as none (0), minor (1), clinically relevant non-major (2), and major (3). FXIII level was measured using HemosIL FXIII antigen assay on ACL TOP 550CTS analyzer. Patients were monitored by activated partial thromboplastin time and bivalirudin level assay. Data were presented as mean ± standard deviation (SD) and interquartile range (IQR). Student t-test and Mann-Whitney U Test were used for statistical analysis with significance at $p<0.05$.

Results: 20 patients (27 cases) were treated with FXIII concentrate. Mean age was 7.5 ± 6.0 years. Most patients required ECMO support for cardiogenic shock (14/20, 70%), and supported with veno-arterial (VA) ECMO (12/20, 60%). Four patients were supported with veno-venous (VV) ECMO, while 4 patients transitioned on VA/VV ECMO during their support course. Some patients had low von Willebrand multimer, but other results of coagulation tests were unremarkable. FXIII concentrate was initially administered at 37 ± 8 units/kg. Pre-FXIII antigen level was measured due to hemorrhagic event for all patients; 7 patients at first week, 7 patients at second week, 6 patients at third or longer weeks from the initiation of ECMO. The median of FXIII antigen level was 41% (IQR 37-46) before FXIII administration. Of note, all patients initially showed less than 50% FXIII antigen. FXIII concentrate was administered at a mean of 13.7 ± 12.9 days after ECMO initiation and at mean initial dose of 37 ± 8 units/kg. Four patients needed the administration of FXIII concentrate to improve bleeding severity repeatedly. FXIII antigen level significantly increased to 68% (IQR 57 - 82) after FXIII administration ($p<0.001$). Bleeding symptoms were improved after FXIII concentrate infusion. The ISTH bleeding score before treatment was a mean of 2.0 ± 0.8%, indicative of mostly clinically relevant non-major bleeding; 6 cases in 6 patients at score 3 (clinically relevant major), 15 cases in 11 patients at score 2 (clinically relevant non-major), and 6 cases in 6 patients at score 1 (mild). All patients improved their bleeding symptoms after FXIII concentrate administration with a significant decrease in ISTH bleeding score to 0.8 ± 0.9, ($p<0.001$). Sixteen patients were successfully decannulated and finally discharged from the hospital.

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Conclusions: Bleeding severity improves after FXIII administration in ECMO patients with initial FXIII level < 50%. Monitoring of FXIII in patients with unexplained ECMO bleeding and FXIII administration at a level <50% is a promising approach and requires further evaluation to mitigate bleeding in ECMO setting.

ID# 20

Mainstem Bronchus Synovial Sarcoma Causing Obstructive Pneumonia

Synovial sarcoma is a rare mesenchymal soft tissue tumor with variable epithelial differentiation that predominantly affects young adults and adolescents. It can arise in or around any tissue, occurring most commonly in the extremities, and rarely in the thoracic and abdominal viscera. Despite its name, the tumor does not arise from synovial cells and has uncertain carcinogenesis. It is, however, well-defined by a t(X;18)(p11.2;q11.2) translocation, forming a fusion protein of SS18 with SSX1, SSX2, or SSX4. Though metastases are present in approximately 50% of cases, tumor behavior and survival are variable, based primarily on stage, age, and location. Herein, we present a case of mainstem bronchus synovial sarcoma leading to obstructive pneumonia of the right lung.

A 38-year-old man presented with a 7 months history of cough, shortness of breath, and hemoptysis. Chest CT showed a mass-like consolidation in the right lung. FISH for defining synovial sarcoma translocation was negative, but was positive with subsequent RT-PCR . The biopsy was read as synovial sarcoma and the patient underwent a subsequent pneumonectomy. Gross examination showed an occlusive mass in the right main steam bronchus, an ill-defined mass with necrotic foci occupying the entire lower and middles lobes, and possible diaphragm invasion. However, microscopic examination demonstrated acute and chronic pneumonia, with no evidence of synovial sarcoma, in all but the bronchial and diaphragmatic sections.

This case demonstrates two critical concepts in tumor diagnosis: need for multiple confirmatory methodologies and evaluation of multifocal tumor. Just as tumors may have aberrant IHC profiles, they may also have this problem with genetic and/or molecular testing; therefore, a single unsuccessful methodology should not by itself exclude a diagnosis. Secondly, the evaluation of separate foci in relation to tumor staging is a point of deliberation, both historically and contemporarily. In many cases, evidence of invasion into prescribed anatomical structures is a sufficient upstaging criterion. For tumors staged based on size alone, like synovial sarcoma, the assessment of multifocal tumor is critical. In our case, given no other sufficient explanation for the foci, the entire distance was treated as tumor and the patient was therefore staged as pT3.

ID# 22

Ectopic or invasive pituitary neuroendocrine tumor: Report of a rare case

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Introduction

Neuroendocrine tumors are uncommon in the sinonasal tract, comprising 3% of sinonasal tumors. Of these, pituitary adenomas make up a significant proportion, usually by direct extension from the sella. However, ectopic pituitary neuroendocrine tumors may present without arising in the sella. We present a case of ectopic pituitary neuroendocrine tumor.

Case Presentation

A 56-year-old man without significant medical history presented with two days of severe, unrelenting headaches. Computed tomography scan of the head showed an expansile soft tissue mass with cystic component centered in the clivus. Magnetic resonance imaging scan gave more detail, showing a 4.7 x 3.6 x 2.8 cm irregular, lytic, and expansile mass. The lesion primarily involved the sphenoid bone centrally, specifically the sphenoid body, sphenoid sinuses, the upper clivus, the sella turcica floor, dorsum sella, planum sphenoidale and dorsum sella, and also involved the left foramen rotundum and bilateral vidian canal and the lesser wing of the sphenoid, with a seen relation into the optic canals without infiltration. It also involved the left medial pterygoid plate. The mass was biopsied and was noted as a friable clival mass with extension into bilateral sphenoid sinuses and rostrum.

Pathologic and Ancillary Findings:

Histologically, the mass was a small, round, blue cell tumor with monomorphic cells with rare rosettes and mitotic figures. Immunohistochemically, the tumor stained positively for vimentin, CD99, synaptophysin, chromogranin, and CD56. They also stained positively for prolactin and a few cells were positive for adrenocorticotropic hormone. Pancytokeratin highlighted focal cells with a dot-like staining pattern. The tumor was negative for SOX10, STAT6, S100, CK20, SMA, ER, and growth hormone. Ki67 had a proliferative index of 1%. The cells had preserved INI. The tumor was positive with FLI1 and PIT1, and negative with SF1. The tumor was diagnosed as ectopic or invasive pituitary neuroendocrine tumor/pituitary adenoma.

Discussion

According to the WHO classification of head and neck tumors, ectopic or invasive pituitary neuroendocrine tumors are tumors composed of anterior pituitary/adenohypophysial cells that arise from the sinonasal tract, rather than direct extension from the pituitary gland itself. These are much rarer than direct extension and careful imaging is required to make this diagnosis and manage care.

ID# 23

Title:
Unlocking The Dual Potential Of EUS-Guided Liver Biopsies: Diagnostic Adequacy In The Setting Of Benign And Malignant Gastrointestinal And PancreatoBiliary Pathology

Background:
Endoscopic ultrasound-guided liver biopsy (EUS-LB) with 19–22-gauge needles is an emerging alternative to traditional liver biopsy. Debate continues over its adequacy and accuracy in diagnosing medical liver diseases (MLD) and mass lesions. The concerns for EUS-LB samples are that they tend to be thinner, more fragmented, and contain fewer portal tracts compared to 16-gauge percutaneous biopsies. This study assesses the clinical utility of EUS-LB, focusing on specimen adequacy and its impact on patient management.

Design:
We retrospectively analyzed 116 EUS-LB procedures performed at our institution between April 2018 and April 2024 for MLD and liver masses. Collected data included patient demographics, clinical indication, lab results, procedural details, and pathologic diagnoses. Biopsies for MLD were obtained with 19-gauge heparin-primed Boston Scientific FNB Acquire needles, while 22-gauge needles were used primarily for liver masses. Rapid on-site evaluation (ROSE) was not utilized. Diagnostic adequacy for MLD was defined as ≥10 portal triads or sufficient material for diagnosis. Tissue was considered adequate for liver masses if it was sufficient for diagnosis, including ancillary workup if needed.

Results:
Of the 116 biopsies, 58 (50%) were for MLD, 8 (6.9%) were post-transplant, and 50 (43.1%) were for liver masses. Among MLD and post-transplant biopsies, 63/66 (95.4%) were adequate. Three cases (4.5%) were inadequate due to excessive fragmentation or low tissue content. In 11 (16.6%) MLD cases, simultaneous ERCP was also performed.

Of the 50 liver masses, 47 were adequate for diagnosis (94%); 4 (8%) were benign and 43 (86%) malignant. 3 (6%) specimens were non-diagnostic of a mass lesion. In 18 (41.8%) cases, simultaneous biopsy of primary tumors (e.g., pancreas, colon, stomach) aided not only in diagnosis but also staging. Simultaneous ERCP was performed in 22 (44%) of these cases.

Conclusion: EUS-LB at our institution has a high diagnostic adequacy rate (95.4% for MLB and 94% for mass lesions). It enables simultaneous tumor staging, evaluation of varices, performance of ERCP and luminal biopsies, depending on the clinical indication. Therefore EUS-LB often reduces multiple procedural needs and provides a minimally invasive option compared to other LB procedures.

ID# 25

Predictors of Fetal Outcome Following Intrauterine Transfusion for Maternal Alloimmunization

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Introduction

Intrauterine transfusion (IUT) is a treatment option for hemolytic disease of the fetus and newborn (HDFN) due to maternal alloimmunization. The aim of this study is to compare fetal outcomes amongst mothers with maternal alloimmunization and identify possible predictive values at our tertiary obstetric center.

Methods

We performed a retrospective review of pregnancies requiring IUT between January 2013 and March 2024 at our center. We then selected for completed pregnancies undergoing IUT for maternal alloimmunization with fetal outcomes (living or deceased). The electronic medical records were reviewed to include maternal demographics, pregnancy history, alloantibodies, antibody titers, and IUT procedure and laboratory data. Statistical analysis was performed based on fetal outcome using the Mann-Whitney U test for comparison of means between the two groups using SPSS version 27 (IBM, NY).

Results

We identified 33 pregnancies (29 patients, 119 IUT procedures) for evaluation with a survival rate of 88%. Anti-D was identified as the primary antibody in 23 pregnancies with a median titer of 256. Concurrent anti-C was present in 11. Other antibodies identified in patients with anti-D included: anti-E (4), anti-G (2), anti-Jka (1), and anti-Jkb (1). Anti-K was the primary antibody in 6 cases with a median titer of 16. Additional antibodies present in patients with anti-K included: anti-E (1) and anti-Jkb (1). Other primary mediators of HDFN in our series included anti-Fya (1, titer 64), anti-c (3, median titer 32), and anti-E (1, titer 64). Notably, three patients developed additional antibodies during IUT including anti-S, anti-Jkb, and anti-C + anti-E. In the fetal demise group, anti-D was present in three patients (two with anti-C) with a median titer of 512. Anti-c (titer 32) and anti-Jka were identified in the last pregnancy with fetal demise. Procedure and laboratory data for IUT are presented in Table 1. There were statistically significant differences between the gestational age at first IUT, presence of fetal hydrops (24% of survivors [n=7] and 100% of fetal demise [n=4], with a p-value of <0.008), initial middle cerebral artery (MCA) velocity in multiples of the mean (MoM), fetal pre-procedure hematocrit, estimated fetal weight per procedure, and post-procedure platelet count. Maternal age and fetal weight at first IUT did not differ significantly between groups.

Conclusions

The risk for fetal demise is higher when IUT is performed at an earlier gestational age and with markers of increased severity of fetal anemia. The significant predictors of fetal demise can be useful for providing prognostic information, patient counseling, and evaluating fetuses throughout treatment. The identification of new antibodies formed throughout IUT therapy is an important area of future research.

ID# 26

Title: Precision Pathology: Unveiling the Role of Interval Sectioning Protocol for Optimal Diagnosis in Pancreatic, Ampullary, and Bile Duct Biopsies

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Introduction: Endoscopic techniques provide advanced diagnostics but face challenges in limited pancreaticobiliary biopsy sampling, including ampullary (AMP), EUS-guided pancreatic (PAN), and spy bite biliary (BD) biopsies. Interval sectioning aims to optimize immunohistochemical stain utility and conserve tissue. This study assesses the impact and utility of interval sectioning protocols on diagnostic precision and tissue conservation at our institution.

Method: In this analysis, cases from a four-month period before and after a protocol change were examined, focusing on AMP, BD, and PAN biopsies. Previously, AMP and BD biopsies involved three H&E-stained slides, while PAN required two H&E-stained and five unstained slides (USS). The new protocol emphasizes consistency with seven slides, featuring H&E staining on levels 1, 4, and 7. Key parameters evaluated included diagnosis, turnaround time (TAT), and the utilization of unstained slides (USS). A 30% USS utilization cutoff was established to justify the continued use of upfront interval sectioning.

Results: A total of 77 cases from the old protocol and 142 cases from the new protocol were reviewed. There is no statically significant difference in the TAT of the old and new protocol (p=0.340). However, there is marked improvement in PAN TAT (4848 min vs 2932 min). Usage rates were different by site: 22.2% (6/27) AMP, 33.3% (7/21) BD, and 35.4% (31/90) PAN.

Conclusion: USS usage was lowest (22.2%) for AMP cases, falling below the 30% threshold for continued upfront interval sectioning justification. Eliminating interval sectioning for AMP would save 116 USS in the new protocol. Upfront interval sectioning remains justified for BD and PAN cases, with USS usage rates of 31.5% and 33.7% respectively. While PAN cases show no cost difference, the new protocol improves specimen visualization, correlates immunohistochemical stains better, and significantly enhances turnaround time (TAT).

ID# 27

Glioblastoma with Prominent Ganglion-Like Cells, a Rare Histopathological Finding

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Introduction: Glioblastoma (GBM), a highly infiltrative glial neoplasm, is the most common malignant primary brain tumor in adults. It is well known that glioblastoma can exhibit variable histologic features. However, the presence of ganglionic differentiation in GBM is rare, with only a few reported cases. Here we report a case of glioblastoma with prominent ganglion-like cells.

Case Presentation: The patient is a 72-year-old woman with past medical history of vertigo, left-sided hearing loss, hypertension, and Hashimoto's thyroiditis. She presented with new onset seizures and decreased strength in the left upper and lower extremities. Brain MRI showed an ill-defined and non-enhancing mass in the right frontal lobe with extension into the corpus callosum.

Pathology: Histopathological examination showed an infiltrative glioma with focal microvascular proliferation without necrosis. Scattered prominent ganglion-like cells were seen focally. Immunohistochemical stains showed that the glioma is positive for GFAP and Olig2. IDH1_R132H is negative, ATRX is retained, and P53 shows strong reactivity in a large subset of tumor cells. BRAF_V600E is negative. The ganglion-like cells were positive for NeuN, neurofilament, and synaptophysin. P53 is also strongly positive in the ganglion-like cells. Methylation tumor profiling showed that the tumor belongs to methylation class “Glioblastoma, IDH-wildtype, RTK2 subtype.” The copy number plot shows a +7/-10 pattern. The final integrated diagnosis is “Glioblastoma, IDH-wildtype, WHO CNS grade 4.”

Conclusions: Here we report a rare case of GBM with very prominent ganglion-like cells. In some instances, the presence of rare, enlarged ganglion-like cells may represent reactive changes; however, the ganglion-like cells in our case are multi-nucleated with strong reactivity of P53, supporting their neoplastic nature. Similar tumors may have been reported in the literature under the name “malignant/anaplastic gangliogliomа/glioneuronal tumor.” Being aware of this rare histological finding can help prevent misclassifying those tumors. The newly available molecular tools (in particular methylome analysis) can also help confirm the diagnosis of glioblastoma in difficult cases.

ID# 28

A t(17;22)(q21;q11.2) Scalp Ewing Sarcoma with No EWSR1 Gene Rearrangement and Harboring EWSR1::ETV4 Fusion

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Abstract

INTRODUCTION

Extraskeletal Ewing sarcoma (EES) is a rare, aggressive malignancy that occurs predominantly in children. Like its osseous Ewing sarcoma (ES) counterpart, EES show sheets of small round blue cells with high nuclear-to-cytoplasmic ratios that show immunohistochemistry findings of strong, diffuse, and membranous CD99 positivity, and diffuse nuclear FLI1 and/or NKX2.2 staining. EES represents less than 10% of all Ewing sarcomas. Similar to ES, EES are characterized by FET::ETS gene fusions including EWSR1::FLI1 in 85% of cases (t(11;22)(q24;q12)) and EWSR1::ERG in 10% of cases (t(21;22)(q12;q12)). EWSR1::ETV1, EWSR1::ETV4, EWSR1::FEV, TAF15::ETV4, EWSR1::SMARCA5, and EWSR1::NFATC2 are less frequently described fusions. We describe the fourth documented case of EWSR1::ETV4 fusion in EES.

CASE PRESENTATION

A 3-month-old boy presented with a progressively enlarging scalp mass in the left occipital area, initially noticed as a small nodule at birth and thought to be a hematoma. Ultrasound suggested a lymphatic malformation or cephalohematoma. Head MRI showed a 7.4 x 4 cm midline and left parietal vertex lesion beneath the galea, abutting the skull with intact overlying fat. The well-circumscribed mass displayed heterogeneous mixed signals, peripheral restricted diffusion, and contrast enhancement with central non-enhancing indicative of necrosis. Whole-body MRI screening showed no evidence of destructive bone lesions. The patient underwent upfront resection and chemotherapy per Texas Children's Hospital's standard, adapted from the COG AEWS1221 Regimen A (VDC and IE), along with reconstructive surgery. Follow-up imaging at 5 and 10 months showed no abnormalities or metastasis, and the patient remains disease-free 16 months post-treatment.

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PATHOLOGY

Biopsy and resection of the lesion showed sheets of small round cells with necrosis and scant cytoplasm with strong diffuse nuclear NKX2.2 and membranous CD99 positivity. The tumor was negative for desmin, myogenin, CD54, S-100, PHOX2B, CD20, CD3, CD34, and TDT, with retained INI-1. FISH testing for EWSR1 rearrangements by breakapart probe did not identify EWSR1 rearrangements on touch preparations from the biopsy. Subsequently, targeted next generation RNA sequencing was performed on the biopsy which identified a EWSR1::ETV4 fusion composed of exon 8 of the 5' gene EWSR1 (NM_013986.3:exon8) on chromosome 22q12.2 and exon 9 of the 3' gene ETV4 (NM_001986.2:exon9) on chromosome 17q21.31. Karyotype was 46,XY,t(17;22)(q21;q11.2)[7]/46,XY[13]. A subclone was identified with an EWSR1 translocation, identifying a breakpoint on chromosome 22 with the EWSR1 probe translocated to chromosome 17, confirmed by FISH analysis of a previously G-banded metaphase.

CONCLUSIONS

EES often presents as a large, soft tissue mass which commonly occurs in the trunk, extremities, and head and neck regions. EWSR1::ETV4 fusion is a rare ETS domain partner, reported exclusively in soft tissue and extraskeletal sites. A specific case involving this tumor in infancy with the EWSR1::ETV4 fusion was described, emphasizing its occurrence in early childhood. Our findings highlight the trend of EWSR1::ETV4 to occur in soft tissue, distinguishing them from the more common skeletal presentations seen with other fusions. We present the fourth case of an EWSR1::ETV4 fusion with extraskeletal involvement, suggesting a possible predisposition of ETV1/4 fusions to non-osseous locations. In the appropriate clinical and histological context, the identification of EWSR1::ETV4 fusion transcripts supports a diagnosis of EES.

ID# 29

PTEN Alteration in ER+ Breast Cancer: Correlative Study of Immunohistochemistry and Next Generation Sequencing

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ABSTRACT

Introduction:

This study is to assess PTEN functional status in ER+ Breast cancer (BC) and evaluate the concordance of PTEN expression by immunohistochemistry (IHC) with *PTEN* gene aberration by next generation sequencing (NGS).

Methods:

372 advanced ER+ BC cases with 146-gene panel NGS were reviewed to assess *PTEN* mutation and copy number aberration. PTEN IHC were performed in selected 70 of 372 cases. PTEN loss by IHC, copy number (CN) alteration and somatic mutations by NGS were examined and association with clinicopathologic features was analyzed. PTEN IHC interpreted as retained (staining in >5% tumor cells), loss (<1%) and equivocal (focal/weak staining 1-5%).

Results:

In 372 ER+ BC cases, 21 (5.6%) had *PTEN* somatic mutations and 9 (2.4%) had CN deletion or partial deletion. In 70 cases with both NGS and IHC tested, NGS detected *PTEN* aberration in 18 (25.7%) including CN deletion or partial deletion in 9, loss-of-function mutation (nonsense,

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frameshift indel or split site mutation) in 5, non-loss-of-function (missense or delins) mutation in 4 tumors. IHC showed PTEN loss in 13 (18.6%), equivocal in 4 (5.7%), and retained in 53 (75.7%) tumors. 92.3% (12/13) tumors with IHC PTEN loss had either CN deletion or loss-of-function mutations by NGS. 98.1% (52/53) tumors with IHC PTEN retained expression had no genetic aberration. 4 IHC equivocal cases exhibited 1 CN deletion, 1 delins mutation, 1 missense mutation, and 1 no aberration.

Conclusion:

PTEN gene aberrations are present in 8.1% of advanced ER+ BC by NGS. PTEN protein loss by IHC correlates with PTEN copy number deletion or loss-of-function mutations. Equivocal PTEN expression warrants further reflex test by alternative assay. Our data supports IHC and NGS testing accurately assess PTEN status.

ID# 30

Investigating Asthma, Inflammatory Bowel Disease and Migraine as a Combined Risk Factor for Endometriosis Using the NIH All of Us Database

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Introduction: Endometriosis is a complicated female reproductive tract disease that impacts around 10% of all women world-wide. Defined as the abnormal appearance of endometrial tissues outside of the uterine cavity, endometriosis not only causes dysmenorrhea and chronic pelvic pain, it also leads to infertility and increased risk for ovarian cancer. However, it has been reported that the diagnosis delay of endometriosis can be up to ten years, which causes a detrimental socio-economic burden for women. Endometriosis has been linked with uterine structural abnormalities, genetic factors, environmental factors and more recently, inflammation and migraine. However, no comprehensive studies have been done to investigate whether the systematic inflammation conditions and/or migraine will increase the risk of developing endometriosis. Here, we aim to examine whether known inflammatory conditions, namely asthma and Inflammatory Bowel Disease (IBD), together with migraine, are risk factors for the onset of endometriosis using the NIH All of Us dataset.

Method: Using All of Us Registered Tier Dataset v8, records that contain sex assigned at birth code, electronic health record (EHR) data and age code were selected for further analysis. Filtering criteria include the sex assigned at birth as female. Cohorts that do not contain confounding factors were generated after such filtering. Next, data were sorted into case and control cohorts defined by whether EHR had records showing asthma, IBD or migraine. Using endometriosis as SNOMED concepts, numbers of individuals with and without these conditions were recorded for both case and control cohorts. Descriptive measurements such as age, race, and ethnicity were also included in the output records. Statistical analysis of odds ratio (OR), P-value and 95% confidence intervals are calculated according to Altman, 1991. Normality distribution was examined by Shapiro-Wilk test. Mann-Whitney U test was used to compare the difference of onset age of endometriosis.

Results: In total, 162520 and 77044 individuals were included in the case and control cohorts, respectively. The control cohort consisted of 1.39% American Indian or Alaska Native, 3.64% Asian, 16.53% Black or African American, 53.31% White, and 25.13% other (due to minimum reporting threshold, these categories were consolidated into other: Another single population; I prefer not to answer; More than one population; None Indicated; None of these; Skip; Native Hawaiian or Other Pacific Islander; Middle Eastern or North African). The case cohort consisted of 1.40% American Indian or Alaska Native, 1.50% Asian, 17.03% Black or African American, 57.11% White, and 22.96% other. Having asthma, IBD or migraine was significantly associated with the occurrence of endometriosis (OR = 2.9437, 95% CI 2.8082-3.0857, P<0.0001). In addition, we report that in patients with asthma, IBD or migraine, the onset time of endometriosis was significantly decreased, with the mean age at condition onset of 44.95 $\pm$ 12.12 years in the control cohort while 42.91$\pm$11.22 years in the case cohort.

Conclusion and future direction: Our results confirmed the comorbidity between asthma, IBD or migraine conditions with endometriosis using the All of Us dataset. In addition, we reported that patients with asthma, IBD, or migraine conditions demonstrated a younger onset age for endometriosis. Our findings affirm the need for further mechanistic studies of the link between endometriosis and asthma/IBD/migraine conditions, but also call for more efforts toward preventative education and awareness of endometriosis in these populations.

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ID# 31

Accurate Diagnosis of Mantle Cell Lymphoma with Cutaneous and Gastrointestinal Involvement: a Rare Case Report

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Introduction: Mantle cell lymphoma (MCL) is a rare, generally aggressive form of non-Hodgkin lymphoma originating from mantle zone B-cells of lymphoid follicles. Nearly all cases have t(11;14) translocations and cyclin D1 overexpression, promoting uncontrolled cell growth. MCL has variable clinical presentation, typically with systemic involvement, including extra nodal sites like the blood, spleen, and gastrointestinal tract. MCL rarely manifests with cutaneous features, complicating differential diagnosis. Here we report a rare case of MCL with gastrointestinal (GI) and skin involvement.

Case Presentation: A 52-year-old male with presumed untreated chronic lymphocytic leukemia (CLL) presented with eight months of progressive chest pain, shortness of breath, fatigue, and unintentional weight loss. Initial labs revealed anemia (hemoglobin 5.2 g/dL) and leukocytosis (white blood cell count 71,200/uL,73.7% lymphocytes). Imaging revealed lymphadenopathy, splenomegaly and gastric thickening. Lost to follow-up, he represented after one month with worsening symptoms and painful bilateral lower extremity swelling. Labs again showed anemia (hemoglobin 4.7 g/dL) and leukocytosis (white blood cell count 74,300/uL,95.7% lymphocytes). Workup included bone marrow (BM) biopsy, flow cytometry, endoscopy with biopsy and punch biopsy of a plaque-like cheek lesion that was discovered.

Pathology: Peripheral blood flow cytometry showed CD5+ kappa-restricted B-cells of small to intermediate size (43% of events). BM biopsy showed hyper cellularity (nearly 100%) and immunohistochemistry highlighted sheets of CD20+, Cyclin D1+ B-cells (~80%). Gastric biopsy also demonstrated Cyclin D1+ cells, indicating gastric involvement by MCL. The skin lesion biopsy exhibited a dense B-cell infiltrate staining CD20+, CD5+, BCL-2+, Cyclin D1+, SOX11+, PAX-5+, and partial CD10+ and BCL-6+, supporting cutaneous MCL involvement.

Conclusions: This case report emphasizes the importance of comprehensive evaluation of MCL for an accurate staging, risk stratification and clinical management. Skin involvement is rare in MCL, and if present, is more commonly seen in an advanced/disseminated stage of the disease.

ID# 32

Toxic Epidermal Necrolysis in the setting of Systemic EBV-positive T Cell Lymphoma of Childhood with Hemophagocytic Lymphohistiocytosis-associated Coagulopathy

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Introduction

Systemic Epstein Barr Virus (EBV)-positive T cell lymphoma of childhood is a rare, often lethal clonal proliferation of EBV-infected cytotoxic T cells occurring in immunocompetent children and young adults. It has a very rapid onset with severe clinical course and is often complicated by hemophagocytic lymphohistiocytosis (HLH), coagulopathy, and multiorgan failure. Rare cases of Steven Johnson Syndrome (SJS) and Toxic epidermal necrolysis (TEN) have been reported in association with HLH and EBV-positive lymphoproliferative disorders.

Case Presentation

This is a 15 year old female who was previously healthy and presented with progressive fever, hepatic dysfunction, cytopenias, and adenopathy. A bone marrow biopsy and concomitant nodal excision demonstrated hemophagocytosis, an aberrant CD3+ CD8+ cytotoxic T cell population, and necrotic nodal tissue. She developed diffuse erythema and desquamation after administration of trimethoprim-sulfamethoxazole and allopurinol with simultaneous profound coagulopathy including massive mucosal and gastrointestinal hemorrhage. A punch biopsy demonstrated epidermal necrosis, subepidermal blistering with EBV-positive lymphocytes in the papillary dermis consistent with TEN. Those medications were discontinued without further cutaneous blistering. Despite several targeted anti-inflammatory therapies, she died weeks later of complications of HLH-induced coagulopathy.

Pathology

At autopsy, there was extensive organ involvement by the atypical EBV+ T cell population and histiocytosis with associated tissue damage involving the mediastinal/hilar lymph nodes, lungs, liver, spleen, small and large intestine, and pancreas. The most significant finding was a markedly dilated large and distal small bowel with 2560 g of predominantly clotted blood in the lumen consistent with significant HLH-induced coagulopathy. The skin showed diffuse patchy hypopigmentation of variably healing skin grossly and intact epidermis with mild perivascular inflammation and focal associated devitalized blood vessels microscopically.

Conclusions

The most common trigger of SJS/TEN is drug-induced secondary to administration of antibacterial sulfonamides, allopurinol, anticonvulsants, and NSAIDS. However, the second most common trigger is infection including bacterial infections (M. Pneumoniae, Group A Beta Streptococci) and viral infections (EBV, CMV, HSV, HHV-6&7, Parvovirus). Unfortunately, the literature of the coexistence of SJS/TEN and HLH is extremely limited. In our case, the patient already had an established diagnosis of EBV-associated HLH based on clinical criteria and lymph node biopsy prior to receiving trimethoprim-sulfamethoxazole and allopurinol and subsequently developing TEN. It is debated whether patients with HLH are at an increased risk of developing TEN based on a common pathway of abnormal lymphocyte stimulation or if the two entities are mutually exclusive. This case draws attention to a drug-induced TEN in the setting of profound immune activation with cytotoxic T cell neoplasia and the importance of understanding this association with lymphoproliferative disorders. It also demonstrates the rare but rapid and profound clinical presentation of Systemic EBV-positive T cell lymphoma of childhood.

ID# 33

Focal Nodular Hyperplasia-Hepatic Adenoma Hybrid Lesion in the Setting of Portal Venous Abnormalities

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Introduction

Hepatocellular nodules can arise as a result of abnormal portal blood flow in the setting of portosystemic shunts (PSS). These nodules include benign entities such as nodular regenerative hyperplasia (NRH), focal nodular hyperplasia (FNH), and hepatocellular adenomas as well as malignant neoplasms such as hepatocellular carcinoma.

Case Presentation

We present an otherwise healthy 18-year-old male with 2-3 days of lower abdominal pain and one-week history of diarrhea. MRI showed multifocal hepatic masses involving the right and left lobes with the largest measuring up to 7.4 cm, favoring FNH or FNH-like lesions. AFP was 2.2 ng/mL.

Pathology

An ultrasound-guided biopsy of the largest mass showed a proliferation of hepatocytes with monomorphic nuclei and diffuse predominantly macrovesicular steatosis throughout the lesion, favoring hepatic adenoma. The patient underwent left hepatectomy and resection of 2 right-sided lesions. The left lobe was almost entirely replaced by multiple confluent nodules with mixed histopathologic features. The nodules included morphology of hepatic adenomas with and without steatosis, FNH, and NRH. No significant cytologic atypia or features of malignancy were seen. Molecular testing did not reveal beta-catenin (*CTNNB1*) or any other high-risk mutations and immunohistochemistry for beta-catenin was negative as well. Peritumor liver showed extensive portal vein abnormalities including thickening, occlusion, dilation, localized cavernoma-like formations and portosystemic shunts.

Conclusions

This case highlights the rare development of multiple benign hepatocellular lesions in the context of PSS. FNH-like nodules in the setting of chronic vascular disorders have been shown to be neoplastic by the presence of beta-catenin activation and *CTNNB1* alterations. As such, appropriate ancillary tests should be used to evaluate these lesions. This case also highlights challenges in the surgical management of multifocal lesions with respect to considerations for a transplant. The patient underwent an ablation procedure following resection and remains clinically stable and is being monitored with repeat imaging as of now.

ID# 34

Development of small molecule modulators of CTLA-4 as a novel strategy for safe and effective immunotherapies

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Antibody-based immune checkpoint inhibitors have revolutionized cancer therapy but often cause severe adverse events and resistance. We sought to develop small-molecule CTLA-4 modulators as safer, equally effective alternatives for both cancer and autoimmune applications, avoiding unwanted protein degradation and immune-related toxicities. To this end, we expressed and purified the extracellular domains of CTLA-4, CD86, and CD80 in human cells, confirming stability and function via dendritic cell binding assays and thermal shift analysis. By harnessing an expansive DNA-encoded library (DEL) of over four billion unique compounds, we uncovered highly specific candidate small molecules targeting CTLA-4, CD80, and their complex. Molecules that specifically bind CTLA-4 could enhance T-cell activation for cancer immunotherapy, whereas those that target the CTLA-4/CD80 complex could heighten immunosuppression and thus prove beneficial in autoimmune conditions. Following a multi-step synthetic effort—encompassing repeated purification and thorough structural validation to confirm each compound's purity and identity—rigorous biophysical assays verified the binding interactions of these molecules, laying a solid foundation for next-generation immunomodulation strategies. These results highlight the importance of small-molecule specificity in designing targeted agents, setting the stage for further optimization and therapeutic exploration.

ID# 35

Diagnostic Challenges in A Case of Soft Tissue Sarcoma with *ZC3H7B::BCOR* Fusion

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Introduction: *BCOR* (BCL6 corepressor) gene located on Xp11.4 encodes a transcriptional corepressor interacting with BCL6. It has also been shown to interact with histone deacetylases and form part of the polycomb repressive complex 1. *BCOR* mutations are being increasingly found in a number of diverse tumors. *ZC3H7B::BCOR* fusions have recently been found in undifferentiated round cell sarcoma, ossifying fibromyxoid tumor, and high grade endometrial stromal sarcoma with the latter being most frequent (81% of *ZC3H7B::BCOR* fusions in a meta-analysis study). These tumors tend to follow an aggressive clinical course. Histologically, *ZC3H7B::BCOR* fusion tumors display a spindle or small round cells with a frequent myxoid morphology. Diagnosis mainly relies on molecular techniques. *BCOR* fusions are usually 5' *BCOR* with 3' partners which leads to BCOR overexpression. Here, we report a case of *ZC3H7B::BCOR* fusion that evades detection by in-house RNA panel and antibody targeting BCOR N-terminus.

Case presentation: An 18-year-old male presented to his PCP with a palpable midline neck mass present for months. Ultrasound showed a 1.7 cm mass. While resection revealed a gross size of 2.8 x 2.5 x 1.5 cm. MRI done three months after the resection revealed a 4.5 x 3.4 x 2.3 cm irregular T1 hypointense, T2 hyperintense heterogeneously enhancing mass in the midline and left para-midline location within the anterior neck, infiltrating the strap muscles, extending from the level of the true vocal cord inferiorly to the thoracic inlet. Multiple abnormal metastatic neck lymph nodes were also identified.

Pathology: The resected mass consists of a tan-pink, multilobular soft tissue mass with scattered myxoid areas. Sections show fibroadipose tissue with a nodular proliferation of oval to spindled cells arranged in a storiform pattern within a myxohyaline stroma and a central zone of relative hypocellularity. There is brisk mitotic activity (46 in 10 contiguous high-power fields) without necrosis, atypical mitotic figures, or significant cellular pleomorphism. The lesion extends at least focally to all six inked margins. The tumor cells are positive for S-100 (weak), CD99 (paranuclear, dot-like), BCL2 (weak), INI1 positive (wildtype pattern), SATB2 (weak), and negative for BCOR, SMA, MSA, Desmin, Myogenin, MYOD1, CD34, pancytokeratin, SOX10, ALK1, MUC4. FISH testing for *SS18* gene rearrangements is negative, and in-house solid tumor fusion panel showed no clinically significant alterations in any of the 81 genes tested, including *CCNB3*. In house solid tumor mutation panel revealed loss of 5' and gain of 3' *BCOR*, loss of *CDKN2A* and *CDKN2B*. Mayo Clinic sarcoma fusion panel detected *ZC3H7B::BCOR* fusion with junction of 5' *ZC3H7B* at exon 10 and 3' *BCOR* at exon 7. Final diagnosis is sarcoma with *BCOR, CDKN2A,* and *CDKN2B* loss.

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Conclusion: This case illustrates two challenging points in classifying sarcoma with rare *ZC3H7B::BCOR* fusions. Clinicopathologically, this tumor does not fit any of the three known entities that harbor *ZC3H7B::BCOR* fusions: high grade endometrial stromal sarcoma, undifferentiated round cell sarcoma, ossifying fibromyxoid tumor. Secondly, the 3' *BCOR* fusion in this case evades detection by a fusion panel targeting the 5' *BCOR* portion and antibody targeting N-terminus.

ID# 36

A Role for Gut Mycobiome and Altered Fungal-Bacterial Interactions in Women with Endometriosis

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Introduction: Endometriosis is a gynecological pathology prevalent in reproductive age women in which the inner uterine wall (endometrium) grows outside as ectopic lesions. The inflammation resulting from these growing implants closely associates with disease severity, causing chronic pain and infertility. Emerging studies have found altered bacterial communities in endometriosis and a causal role for gut bacteria in endometriosis. However, the role of the gut mycobiome *i.e.,* the fungal component of the microbiome in endometriosis is a current knowledge gap that needs to be addressed.

Methods: We utilized the stool samples from women with endometriosis and performed 16S rRNA and ITS2 gene sequencing for profiling the gut bacteria and fungi, respectively. By performing an integrated analysis, we studied the co-occurring relationships between fungi and bacteria. In addition, we determined the microbial interactions with the host and identified the bacterial taxa as microbiome-associated host genetic variants in endometriosis. Further, we performed in-vivo studies in mouse model of the disease to understand the effect of gut fungal depletion on the progression of endometriosis.

Results: We found that, in addition to bacteria, the gut fungal communities are also altered in women with endometriosis. The integrated analysis revealed that the interactions between gut fungi and bacteria are also altered during endometriosis. Through study of these interactions, we highlight an important role of fungi as underlying regulators of the disease. Experimentally, we demonstrate that the progression of endometriosis in mice is significantly impeded by the depletion of fungi, revealing a significant role for the gut mycobiome in endometriosis.

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Conclusions: Our results highlight the positive- and negative- co-occurrence relationships shared between bacteria-fungi, bacteria-bacteria and microbes-host in the disease pathogenesis. These findings suggest that the bacterial-fungal interactions must be contemplated when designing microbiome-based therapeutic strategies using antifungal agents.

ID# 37

Phyllodes Tumor of the Breast in Hispanic Population: A Clinicopathological Study of 226 Cases

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Introduction: Breast Phyllodes tumor (PT) is a fibroepithelial tumor and is graded as benign, borderline, or malignant histologically 1. It is rare that it accounts for 2.5 % of fibroepithelial lesions and 0.3–1% of all primary breast tumors 2. There are few reports on the race-related difference on clinicopathological characteristics without consensus.

We analyzed clinicopathological characteristics and outcomes of PT patients, comparing Hispanics with non-Hispanics to elucidate the race-difference.

Methods: We analyzed 226 PT patients with diagnosis of PT in surgical excision specimen on Institute database retrospectively at Harris Health Ben Taub General hospital, Houston, Texas, between 2005 and 2024.

Results: Among 226 PT patients, 177 (78.3 %) were Hispanic and 49 (21.7 %) were non-Hispanic. 142 (80.2%) of 177 Hispanic were diagnosed with benign, 13 (7.4%) with borderline, and 22 (12.4%) with malignant PT. Non-Hispanic were diagnosed with significantly more malignant PT (20.4%).

In malignant PT, all patients received surgery primally, including 50.0 % of mastectomy in Hispanics and 70.0 % in non-Hispanics, with negative margins of 86.4 % and 80.0 % respectively. 13.6 % of Hispanics and 4.1 % of non-Hispanic received radiation and/or chemotherapy. In Hispanics, recurrence and metastatic rates were lower (9.0 % and 18.1 % respectively), and overall survival (OS) rate was better (86.4 %) compared to non-Hispanics (10.0%, 40.0 % and 67.0 % respectively).

OS and disease-free survival (DFS) in patients with malignant PT were significantly ($p<0.05$) correlated with the absence of heterologous components and the tumor pathologic stage, while not correlated with negative surgical margins or radiation therapy.

Conclusion: Hispanic PT patients are younger and tend to have fewer malignant type, and lower recurrence and metastatic rates, and better overall survival, compared to those of non-Hispanic patients. However, the difference was small and not significant. Overall survival of malignant PT is correlated with the absence of heterologous components and the tumor stage. The workup and the treatment of women with PT should not differ according to race.

ID# 39

Childhood Acute Myeloid Leukemia with CEBPA mutations

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Introduction: AML with *CEBPA* mutation constitutes approximately 5% of AML cases in children and 5–11% of AML cases in adults. *CEBPA* (CCAAT enhancer binding protein alpha) encodes a transcription factor that contains a basic leucine zipper (bZIP) domain and recognizes the CCAAT motif in the promoters of target genes. The *CEBPA* gene and the bZIP transcription factor that it encodes is essential for myeloid lineage commitment and regulates myeloid differentiation and stem/progenitor cell function. Acute myeloid leukemia (AML) with *CEBPA* mutation is a subtype of AML that have favorable prognosis, per current guidelines, and may be characterized by either biallelic *CEBPA* mutations (bi*CEBPA*), or single mutations within the C-terminal basic leucine zipper (bZIP) domain (smbZIP*CEBPA*), the latter typically being in-frame insertion-deletion mutations. Here we report a case with mutations in the N' and C' terminus, within the bZIP domain, that supports acute myeloid leukemia with biallelic CEBPA mutations.

Case Presentation: Patient is a 14-year-old female with history of newly diagnosed acute myeloid leukemia. The patient presented with upper respiratory infection, fatigue, fever, and rash with blisters in the mouth and tongue. On routine laboratories studies the patient presented leukocytosis (263.6X10*3/uL).

Pathology: Flow cytometry study was performed and showed blasts (94%) marked as myeloid with granulocytic differentiation, consistent with acute myeloid leukemia. Due to the prominent circulating peripheral blast count treatment was initiated and a bone marrow biopsy was performed at end of induction therapy. The bone marrow biopsy showed blasts (0.3%) marked as myeloid with granulocytic differentiation as identified by flow cytometry analysis, consistent with residual acute myeloid leukemia. A Heme DNA/RNA combined panel was ordered and identified A **p.Thr310dup** in-frame insertion variant at a variant allele fraction (VAF) of 43% and a **p.Ala113fs** frameshift variant at a VAF of 46% in the ***CEBPA*** gene. The **p.Thr310dup** in-frame insertion variant is located in the C' terminus and within the bZIP domain of the CEBPA gene. The **p.Ala113fs** frameshift variant is located in the N' terminus and between the transactivation domain (TAD1) and TAD2 domain of the CEBPA gene. The presence of

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these variants, particularly the **p.Thr310dup** in-frame insertion variant within the bZIP domain of CEBPA gene categorizes this leukemia as acute myeloid leukemia with CEBPA mutation per WHO Classification of Tumours (Haematolymphoid Tumours, 5th edition). Acute myeloid leukemia with CEBPA mutation confers a favorable prognosis, particularly when the mutation is found within the bZIP domain, compared to the wild type acute myeloid leukemia or CEBPA mutation located in the N' terminus and TAD domains of CEBPA gene.

Conclusion: The mutation profile presented in this case is consistent with a diagnosis of AML with biallelic *CEBPA* mutation. In 5-10% of cases, bi*CEBPA* may reflect an underlying germline *CEBPA* mutation, typically the N-terminal frameshift variants. Therefore in such cases, genetic counseling and germline testing is recommended.

ID# 40

Development and Validation of a Metagenomic-Based Method for Pathogen Identification in Pediatric Patients

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Introduction

Metagenomic sequencing (mNGS) is increasingly utilized in clinical settings due to its ability to detect pathogens, including those present in low abundance. We developed Sequence-based Pathogen Identification (SEQ-ID), a diagnostic test for microbial (bacterial or fungal) pathogens tailored for the unique patient population at Texas Children's Hospital, which includes pediatric patients who may be immunocompromised and at greater risk for opportunistic infections. Here, we present SEQID's performance compared to culture-based assays and targeted sequencing.

Methods

Parallel aliquots were obtained from specimens collected from pediatric patients at Texas Children's Hospital, where external microbial (bacterial or fungal) sequencing from a reference lab was ordered. A total of 186 pediatric samples (73 soft tissue, 46 body fluid, 8 bone, 48 bronchoalveolar lavage, 11 abscess) with sufficient volume for both routine diagnostic testing and this study were collected. Nucleic acids were extracted using the Qiagen DNeasy PowerSoil Pro Kit, Revvity NEXTflex Rapid DNA-Seq kit for sequence library preparation, and Illumina platform for sequencing. Sequences were host-filtered, quality-trimmed, and classified using sra-human-scrubber, multitrim, and KrakenUniq, respectively. Pathogen identification was based on read counts and microbial distribution.

Results

Total WGS microbial read counts ranged from 0 to ~256,000. A microbial community was identified in 49 samples, with a potential pathogen(s) identified in 43 samples and expected microbiota identified in 6 samples. SEQ-ID results agreed with reference lab testing or culture 85% of the time. Discrepancies were due to low read counts below reporting thresholds. SEQ-ID identified additional pathogens and co-infections not detected by reference lab testing. Turnaround time for SEQ-ID was ~5-7 days, compared to ~2 weeks for reference lab testing.

Conclusions

SEQ-ID is a robust method for pathogen identification in pediatric patients, offering high sensitivity and specificity for bacterial and fungal detection. It enhances diagnostic capabilities in pediatric infectious disease settings by providing an untargeted and culture-independent reliable alternative to the current reference laboratory strategy.

ID# 43

An Unusual Case of EBV-associated Gastric Carcinoma of the Stomach: A Case Report and Literature Review

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Introduction: Epstein-Barr Virus (EBV) is an established cause of various types of lymphoma and nasopharyngeal carcinoma. EBV has a significant role in the pathogenesis of gastric carcinoma with lymphoid stroma (GCLS), also known as medullary carcinoma. Tagged by the unique clinicopathological presentation and favorable prognosis, EBV-GCLS is a distinct type of gastric cancer with characteristic genetic profiles and a very low risk of lymph node metastasis.

Case presentation: A middle-aged man presented with burning epigastric pain, hematemesis, and rectal bleeding for a week. The patient also had a history of increased pallor, fatigue, and weight loss for the last 6 months. CT abdomen and endoscopy revealed gastric wall thickening with a 25 cm cratered, irregular, malignant appearing ulcer involving the body and greater curvature of the stomach. Endoscopic biopsies and later on subtotal gastrectomy were performed.

Pathological and ancillary findings: The biopsies from the ulcerated mass showed ulcerated invasive adenocarcinoma with proficient MMR and negative HER2. After completing eight cycles of neoadjuvant therapy (FLOT), the patient underwent surgery. The specimen revealed tumor cells were arranged in irregular sheets, trabeculae, and tubular patterns uniformly distributed throughout the lymphoid stroma. In situ hybridization for EBER shows diffuse and intense expression in all tumor cells and positivity for AE1/AE3. A total of 35 lymph nodes were examined without metastatic carcinoma.

Discussion: EBV-GCLS has typical genetic profiles with a frequent mutation in PIK3CA and amplification of JAK2 and PDL1. Therefore, it shows unique features including a male predominance, proximal stomach location, and a better prognosis. It could be challenging for pathologists to diagnose it on biopsy as many other types of gastric cancers may have associated inflammation. In clinical practice, patients with early EBV-GCLS might be good candidates for minimally invasive surgery because of the low rate of lymph node metastasis, and for advanced cases, immunotherapy might be helpful based on PD-L1 overexpression. In conclusion, EBV can serve as a biomarker in GCLS and might be helpful for the future development of new potential therapeutic targets.

ID# 44

Donor-Derived Myeloid Sarcoma Following Cord Blood Stem Cell Transplantation: a Rare Case Presentation and Review of Literature

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Introduction: Myeloid sarcoma is a rare extramedullary presentation of acute myeloid leukemia (AML) characterized by variable clinical presentation involving the skin, lymph nodes, GI tract, and other anatomical sites. Nearly all cases of myeloid sarcoma are associated with concurrent AML; however, some cases of disease occur in isolation or manifest as relapse of a previously treated AML. Molecular abnormalities in myeloid sarcoma largely recapitulate those found in AML, and appropriate molecular characterization is needed to inform therapeutic strategies. Here we report a unique case suspicious for a donor-derived myeloid sarcoma in a patient with history of AML in remission following cord blood stem cell transplantation.

Case Presentation: An 11-year-old male with a history of FLT3-ITD+ AML in remission following cord blood stem cell transplantation presented with respiratory failure several months after transplant. The patient's course was complicated by hepatic veno-occlusive disease (VOD), posterior reversible encephalopathy syndrome (PRES), and new cutaneous lesions on the back and shoulders. A workup for suspected AML relapse included punch biopsy of a right shoulder skin lesion, bone marrow (BM) examination with flow cytometry, and targeted sequencing of both the tissue and BM specimens. The patient remained in critical condition after presentation and died of acute on chronic hypoxemic respiratory failure.

Pathology: Punch biopsy of the skin lesion revealed infiltrating leukemic blasts and confirmed the diagnosis of myeloid sarcoma, consistent with extramedullary relapsed AML. Strikingly, BM pathology and flow cytometry were negative for abnormal blasts or lymphoid populations and sequencing and engraftment studies of the BM specimen were consistent with 100% donor cells. Targeted sequencing of the skin biopsy did not reveal evidence of the FLT3-ITD, WT1, or UBTF variants observed in the original diagnostic AML BM. Instead, only variants of uncertain significance were detected in the tissue. One set of variants matched the most recent BM specimen, with high variant allele fractions (VAFs >40%), however variants originally attributed to the patient were present only at low VAFs (<7%). Together, these findings are highly suggestive of a donor-derived myeloid sarcoma.

Conclusions: Myeloid sarcoma is a rare presentation of AML requiring concurrent analysis of bone marrow to determine areas of relapse. Donor-derived disease is rarely reported in the literature and should be evaluated in patients with suspected relapse in the setting of incongruous molecular features. Importantly, distinguishing between relapsed AML and a new diagnosis is essential to guide treatment strategies and determine prognosis. This case report demonstrates the value of comprehensive evaluation of multiple tissues (e.g. bone marrow, myeloid sarcoma tissue) in the diagnosis and treatment of myeloid sarcoma.

ID# 46

Integrated Molecular and Clinicopathologic Analysis of Myofibroma Tumors Expands Spectrum of *PDGFRB* and *NOTCH3* Mutations

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INTRODUCTION

Infantile myofibroma (IM) is a rare benign pericytic tumor of children caused by inherited or acquired *PDGFRB* mutations and, less commonly, *NOTCH3* mutations or *SRF::RELA* fusions. We report on a series of 14 IM patients revealing novel *PDGFRB* and *NOTCH3* mutations and potential mosaicism as a genetic mechanism.

METHODS

Institutional genomic database was queried for *PDGFRB*-mutated IM tumors and/or related pericytic tumors identified through clinical next generation sequencing (NGS) tests between 2018 and 2024. Clinical records, histopathology, and molecular results were reviewed. In select patients with an available specimen, tumors were also assessed for genetic mosaicism (n=1) or *cis/trans* phase of double *PDGFRB* mutants (n=1) through NGS or allele-specific RT-PCR.

RESULTS

A total of 14 *PDGFRB*-mutated IM or IM-like tumors (8M, 6F) were identified in patients between ages 0.1-15 years (median age 1.4). Lesions were solitary (n=7) or multicentric (n=7), presenting in soft tissue (n=8), bone (n=4), or viscera (n=2). Histologically, 12/14 lesions were smooth muscle actin (SMA)-positive spindle cell neoplasms with infrequent mitoses of 0-4 per 10 high-power fields (HPF). However, one lesion showed increased mitoses (10 per 10 HPF) and another lacked SMA immunoreactivity. A total of 21 activating *PDGFRB* variants were detected in the 14 tumors, including 7 tumors with double

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mutations. The *PDGFRB* variants were localized to known mutational hotspots in the tyrosine kinase domain (TKD, n=10; p.N666K/T/S n=9; p.D850V n=1), juxtamembrane domain (JMD, n= 8; p.R561C n=3; p.Y562C/D n=2; p.Y589C n=1; indel variants n=2), and transmembrane domain (TMD, n=3). All 3 TMD variants were single mutants, including 2 novel variants (p.A537_I538delinsPN, p.A537_I538delinsDN). In contrast, JMD variants, including 1 novel variant (p.V568_I569del), were present as double mutants together with TKD variants in 7/8 tumors. In one double mutant tumor (IM2), analysis revealed the TKD and JMD mutations to be *in cis*. Pathogenic *PDGFRB* variants were absent in the germline in 4/14 patients analyzed. In a germline-negative patient with multicentric lesions (IM9), the same TKD mutation was detected in discrete lesions, suggesting potential somatic mosaicism. Finally, a novel *NOTCH3* p.L1611_L1626dup variant was detected in a *PDGFRB*-single-mutant bone lesion (IM8), suggesting potential cooperativity. In histologically atypical tumors with high mitotic rate or lack of SMA immunoreactivity, detection of *PDGFRB* mutations were diagnostic.

CONCLUSIONS

We report novel somatic variants in *PDGFRB* and *NOTCH3*, and a possibility of somatic mosaicism in 1 patient with multicentric disease, in a series of 14 IM patients. 2/3 novel variants in *PDGFRB* were single hits affecting the TMD, a domain less commonly reported to be mutated. As reported previously, *PDGFRB* double hits were frequently detected (50% of tumors) and confirmed to arise *in cis* in 1/7 tumor analyzed. Interestingly, JMD variants of *PDGFRB* were preferably associated with double hit tumors (7/8, 87.5%) and *PDGFRB* single hit mutations were predominantly in TKD or TMD in 6/7 tumors (85.7%). Molecular testing for *PDGFRB* mutations provides diagnostic utility for IM with atypical morphology. Our report expands the spectrum of reported *PDGFRB* variants in IM.

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TABLE

Table. Summary of clinical, pathologic, and molecular findings.

Case	Age	Gender	Location	Morphology	SMA IHC	Multi-focality	Germline Mutations	*PDGFRB* TKD Mutation	*PDGFRB* JMD Mutation	*PDGFRB* TMD Mutation	*NOTCH3* Mutation
IM1	2M	Male	Chest wall	High mitotic rate	+	Yes	No	p.N666K	p.V568_I569del[1]		
IM2	2M	Female	Arm	Spindle cell neoplasm	+	Yes	Unknown	p.N666K[2]	p.R561C[2]		
IM3	4M	Male	Lung	Spindle cell neoplasm	–	Yes	Unknown	p.N666S	p.R561C		
IM4	2Y	Female	Mandible	Spindle cell neoplasm	+	No	Unknown	p.N666K	p.Y589C		
IM5	2Y	Female	Scalp	Spindle cell neoplasm	+	No	Unknown	p.N666T	p.Y562D		
IM6	2M	Male	Arm	Spindle cell neoplasm	+	Yes	No	p.D850V			
IM7	5M	Female	Lung	Spindle cell neoplasm	+	Yes	No			p.A537_I538delinsPN[1]	
IM8	12Y	Male	Tibia	Vascular lesion	+ focal	No	Unknown	p.N666K			p.L1611_L1626dup[3]
IM9	7Y	Male	Forearm	Spindle cell neoplasm	+	Yes	No	p.N666K			
IM10	8M	Male	Scalp	Spindle cell neoplasm	+	No	Unknown		p.K567_Y579delinsRTNP		
IM11	2M	Female	Chest wall	Spindle cell neoplasm	+	Yes	Unknown	p.N666T	p.R561C		
IM12	15Y	Female	Skull	Spindle cell neoplasm	+	No	Unknown	p.N666K	p.Y562C		
IM13	11Y	Male	Skull	Spindle cell neoplasm	+	No	Unknown			p.I538_L539insR	
IM14	2Y	Male	Skull	Spindle cell neoplasm	+	No	Unknown			p.A537_I538delinsDN[1]	

[1]Novel *PDGFRB* variants. [2]Double *PDGFRB* mutations *in cis*. [3]Novel *NOTCH3* variant.

M, months; Y, years; IHC: Immunohistochemistry; TKD, kinase domain; JMD, juxtamembrane domain; TMD, transmembrane domain

PDGFRB: NM_002609.3; NP_002600.1; *NOTCH3*: NM_000435.2; NP_000426.2

ID# 47

Defining the Role of RIOK1 in Endometrial Cancer

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Introduction

Endometrial cancer is the most common gynecological cancer in the United States. With the current standard of care, the overall five-year survival rate for women with late-stage endometrial cancer is only about 15%. To identify new therapeutic targets for endometrial cancer, our group performed a screen of Clinical Proteomics Tumor Analysis Consortium (CPTAC), The Cancer Genome Atlas (TCGA), and DepMap data to identify kinases that are both i) required for endometrial cancer cell survival and ii) associated with increasing tumor grade. From this analysis, our group pinpointed Rio Kinase 1 (RIOK1), which is an atypical protein kinase, as well as an understudied “dark kinase.” Few studies have characterized its functions, although it has been found that RIOK1 expression may be linked to oncogenesis and metastasis. Preliminary studies in our lab have also indicated the differential expression of an uncharacterized short RIOK1 isoform that increased in healthy endometrial samples compared to those of the endometrial cancer tumors. Our goal is to define the role of RIOK1 in endometrial cancer and determine functional differences between the long and short RIOK1 isoforms.

Methods

To assess *in vitro* effects of RIOK1 expression on endometrial cancer cell lines, we have performed cell viability, soft agar colony formation, and invasion/migration transwell assays on TEN and HHUA cell lines stably expressing a doxycycline-inducible shRIOK1 vector. Similar *in vitro* studies in primary patient-derived organoids are currently ongoing to assess the effect of RIOK1 knock-down on viability in endometrial cancer organoids. To investigate the differential effects of the short and long RIOK1 isoforms in endometrial cancer cell lines and patient-derived organoids, we will generate cell lines stably expressing either short or long RIOK1 via lentiviral transduction. Functional redundancies will be assessed through *in vitro* cell line assays, and independent pathway activation of these two isoforms will be identified using a phosphoproteomic array.

Results

In vitro assays in TEN and HHUA cells demonstrate that RIOK1 knock-down reduces cell viability, migration, invasion, and soft agar colony formation. Interestingly, knock-down of RIOK1 in 2D cell lines is associated with changes to F-actin architecture, with fewer actin projections on the leading edge of shRIOK1 cells. Preliminary cell viability assays in primary patient-derived organoids from endometrial cancer tumors similarly reveal reduced cell viability following RIOK1 knock-down. When comparing RIOK1 protein expression between endometrial epithelial organoids from healthy patients to those derived from endometrial tumors, the healthy organoids almost exclusively express a short, uncharacterized isoform of RIOK1, while the cancer organoids express varying amounts of both the short and long isoforms.

Conclusions

Given the association between RIOK1 expression and cancer progression in other cancer models, as well as its potential role in actin dynamics, the primary goal of this project is to define the role of RIOK1 as a driver of endometrial cancers. Understanding the downstream effects of RIOK1, and its two distinct isoforms, in endometrial cancer will inform future investigations into its use as a potential therapeutic target in treating this disease.

ID# 48

C/EBP-β Acts as a Rheostat Switch Controlling GATA3 Driven Dendritic Cell CTLA-4 Expression in Response to Th-polarizing Signals

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Dendritic cells (DC) are the most powerful antigen-presenting cells due to their unique ability to elicit de novo adaptive immune responses. DCs are a promising cell therapy modality to combat cancer and attenuate autoimmune disease. However, their clinical use is hampered by an incomplete understanding of the molecular mechanisms by which DCs regulate immune activation and polarization. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is an immune inhibitory molecule well characterized in lymphoid cells and is the target of FDA-approved immune checkpoint inhibitors. Our lab has found that DC CTLA-4 is upregulated in mature DCs and plays an important role in downstream T-cell activation. In vivo mouse models have shown that siRNA knockdown of CTLA-4 in a B16 mRNA-loaded DC vaccine enhances survival and tumor control, highlighting the need to understand DC CTLA-4 regulation. The transcriptional mechanisms controlling DC CTLA-4 remain uncharacterized. C/EBP-β and GATA3 are transcription factors (TFs) known to regulate T-cell CTLA-4 expression, but their role in DCs is unknown. My thesis work suggests that C/EBP-β acts as a suppressor while GATA3 serves as an activator of DC CTLA-4 transcription. C/EBP-β is highly expressed in immature DCs and is downregulated upon maturation, inversely correlating with CTLA-4 expression. ChIP assays confirm direct binding of C/EBP-β to the CTLA-4 promoter in immature DCs. siRNA knockdown of C/EBP-β increases CTLA-4 mRNA and protein levels, supporting its suppressive role. Conversely, GATA3 and CTLA-4 are upregulated in mature DCs and further induced by Th2-skewing signals (SEB, Pam3CSK4, DKK1). GATA3 siRNA knockdown reduces CTLA-4 expression, and ChIP assays confirm direct binding of GATA3 to the CTLA-4 promoter, supporting its role as an activator. Interestingly, C/EBP-β appears to regulate GATA3 expression itself, as C/EBP-β knockdown leads to increased GATA3 mRNA and protein. This suggests that C/EBP-β functions as an upstream rheostat switch, tuning DC CTLA-4 expression by both directly suppressing CTLA-4 transcription and modulating GATA3 levels. Further experiments will expand on DC C/EBP-β and GATA3 roles in CTLA-4 regulation: luciferase assays to demonstrate TF specificity, DC–T-cell co-culture assays to assess functional relevance, ChIP-seq to map the broader transcriptional network governing DC CTLA-4, and in vivo mouse models to evaluate their roles in a DC vaccine model. Completion of this work will elucidate the regulatory interplay between C/EBP-β, GATA3, and CTLA-4 in DCs, providing insights into DC immune regulation. These findings could inform novel DC-based cell therapy strategies, including transgenic modulation of key transcriptional regulators, to enhance therapeutic efficacy in cancer patients.

ID# 49

NK/T Cell Lymphoma Presenting As Adrenal Mass: A Case Report of an Extremely Rare Entity

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Introduction: Extranodal Natural killer/T-cell lymphoma (ENKTL) is an aggressive Epstein–Barr virus (EBV) associated non-Hodgkin's lymphoma. EBV infection and subsequent genetic alterations in infected NK or T cells are central to NKTL development. It is common in Asia and Latin America and typically arises in the nasal cavity with extra-nasal involvement including the skin, gastrointestinal tract, and bone marrow. However, primary involvement of the adrenal gland is very rare and represents an unusual clinical presentation.

Case Presentation: A 61-year-old Hispanic male presented with dyspnea, chest pain, and abdominal pain for 3 weeks. A CT scan revealed bilateral solid adrenal masses measuring 8.0 x 4.2 cm on the right and 8.2 x 5.7 cm on the left, along with hepatosplenomegaly, and ground-glass nodules in the lungs. A CT-guided biopsy of the left adrenal gland mass and flow cytometry of bronchoalveolar lavage (BAL) was performed.

Pathology: The left adrenal mass biopsy showed a neoplastic lymphoid infiltrate, which is positive for CD2, CD3, CD56 (strong) and negative for CD5, CD8, CD10, CD20, CD30, ALK, and TdT by IHC. EBER ISH is positive in most of the tumor cells. Flow cytometry (FCM) of the BAL also identified a significant abnormal NK/T-cell population which was positive for CD2, CD56, and negative for surface CD3, CD5, CD 16. The peripheral blood FCM was negative. The patient was diagnosed with ENKTL and was treated with the "SMILE" protocol (dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide). Unfortunately, the patient passed away 3.2 months after diagnosis.

Conclusions: Primary ENKTL of bilateral adrenal glands is rare with aggressive clinical behavior and often leads to adrenal failure and death. There have only been a few cases reported worldwide and the highest reported survival was more than one year. Rare location of the disease often leads to a misdiagnosis of adrenal carcinoma or metastasis. Careful morphologic assessment with immunophenotypic study and EBER in situ hybridization is needed to confirm the diagnosis. This unique case report will add to the scant literature on this tumor. Further studies and clinical experience are needed for better understanding and development of new effective therapies for this rare disease.

ID# 51

Dendritic cell-intrinsic AIMp1 governs adaptive immune polarization and anti-tumor immunity through NMNAT1

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Introduction

Dendritic cells (DCs) are responsible for driving adaptive immunity by interpreting environmental signals and then initiating and polarizing the downstream adaptive immune response to most effectively combat pathogens. However, in cancer DCs can fail to promote effective adaptive immune polarization and anti-tumor immunity due to a lack of strong polarizing cues and because tumors actively antagonize DC functions. Therefore, there is urgent need to define DC-intrinsic signaling mechanisms that regulate their function in cancers to promote cellular immunity, which can be targeted by future cancer therapeutics. Recent work suggests DC-mediated anti-tumor immunity relies on DC-intrinsic expression of a subunit of the multi-aminoacyl tRNA synthetase complex (mARS), called AIMp1. Therefore, we investigated the manner through which DC-intrinsic AIMp1 mechanistically regulates cellular signaling to promote anti-tumor immunity.

Methods

We first interrogated the immunological profile of DC in mice with a conditional DC-intrinsic AIMp1 knockout by comparing tumor growth and tumor infiltrating lymphocyte (TIL) populations between *Itgax*$^{Cre+}$*Aimp1*$^{fl/fl}$ and *Itgax*$^{Cre-}$*Aimp1*$^{fl/fl}$ mice challenged with B16-LCMVgp melanoma tumors. Given the dual role of AIMp1 as both a pro-inflammatory cytokine and intracellular signaling protein, we then establish the relative contribution of AIMp1 cytokine versus intracellular signaling. Additionally, mass spectrometry was used to identify AIMp1-interacting proteins and metabolic differences between AIMp1-deficient and AIMp1-sufficient murine DC.

Results

We observed increased B16-LCMVgp tumor growth in *Itgax*$^{Cre+}$*Aimp1*$^{fl/fl}$ mice compared with *Itgax*$^{Cre-}$*Aimp1*$^{fl/fl}$ mice. A comparison of TIL populations between those two groups of mice revealed no differences in total or tumor-specific CD8$^+$ T cell populations, but DC-intrinsic loss of AIMp1 was associated with a significant reduction in total CD4+ T cells. Moreover, DCs strictly secreted AIMp1 within extracellular vesicles (EV) during maturation, indicating DC-intrinsic AIMp1 primarily functions as an intracellular signaling effector molecule. Immunoprecipitation-mass spectrometry confirmed AIMp1 as a member of the mARS complex but also revealed a novel interaction with the NAD$^+$-producing nuclear enzyme NMNAT1. Subsequent metabolic analysis of AIMp1$^{-/-}$ DC demonstrated they have suppressed levels of cellular NAD$^+$ along with other core metabolites in the citric acid cycle (TCA).

Conclusions

The results of our initial tumor modeling suggest that the loss of DC-intrinsic AIMp1 led to increased tumor growth by impairing tumor infiltration by CD4+ T cells. After further investigating the mechanisms by which AIMp1 regulates DC signaling, our results suggest AIMp1 primarily functions as an intracellular signaling molecule that can be exported within EVs during maturation. Upon further investigation of intracellular AIMp1-interacting proteins, the binding of AIMp1 with NMNAT1 suggests AIMp1 may functionally regulate cellular NAD$^+$ production. That notion is supported by our metabolomics data showing that the loss of DC-intrinsic AIMp1 causes a loss of cellular NAD$^+$ and other core TCA cycle metabolite that are critical for cellular energy production and immune polarization during DC maturation. Taken together, these data underscore the importance of DC-intrinsic AIMp1 as a regulator of anti-tumor immunity and support further research into understanding its mechanisms of cellular regulation.

ID# 52

Spectrum of Liver Histology Findings in Patients with Transient Abnormal Myelopoiesis (TAM)

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Introduction:
Transient abnormal myelopoiesis (TAM) is characterized by the presence of increased peripheral blasts with acquired mutations in *GATA1* in a neonate with Down syndrome (DS). Most cases resolve spontaneously without sequelae, but there is a risk of progression to myeloid leukemia and increased early mortality due to liver failure. Detailed studies on liver histology are limited and interpretation is often challenged by other co-existing conditions such as prematurity, total parenteral nutrition, congenital heart disease (CHD), biliary atresia (BA), etc. We aim to characterize the liver histopathologic changes in patients with TAM.

Methods:
Pathology database was queried to identify patients with TAM with sampled liver tissue; from 2006 to 2022. All available pathology material was studied and electronic medical records were reviewed. Seven patients with DS and TAM were identified; with 10 liver samples - biopsies (n=5) and autopsies (n=5). Age at sampling ranged from 1.25 to 6 mo, mean 2.9 mo with M:F 4:3. Liver failure was a significant cause of demise in 4 out of 5 patients all of whom had resolution of peripheral blasts. Both surviving patients did not have liver failure. Only 1 had significant CHD with potential effect on liver. Conjugated bilirubin (c-bil) was elevated at the time of all except one sample. Liver biopsy was performed either to rule out BA or for persistent liver panel elevation after the resolution of peripheral blasts.

Results:
Pericellular fibrosis, pericentral fibrosis, and giant cell change were the most frequent histopathologic abnormalities (80% each) followed by hepatocellular/canalicular cholestasis (70%), central vein obliteration (60%), portal/periportal fibrosis (50%), and ductular reaction/bile plugs (40%). Some of these findings are in keeping with published reports of hepatic changes in TAM. There were no peripheral blasts at the time of any sample (biopsy/autopsy). Only 2 out of 10 samples showed tissue extramedullary hematopoiesis along with atypical megakaryoblasts; of whom one was tested (patient 6) and showed p.Met1 frameshift variant in the *GATA1* gene at a variant allele frequency of 23.7% by targeted Next-Gen sequencing of the formalin-fixed paraffin embedded liver biopsy, confirming direct hepatic involvement by TAM in the absence of peripheral blasts. Additional immunohistochemistry and molecular testing are underway.

Conclusions:
Our series extends the hepatic histology in TAM to include a mixed pericellular and perivenular pattern of fibrosis with frequent central vein obliteration. It also highlights that about 40-50% of patients can show portal changes mimicking BA, posing a diagnostic challenge. The high mortality in our series is consistent with the known contribution of liver failure.

ID# 53

Unveiling Rarity: A Unique Case of Mixed Acinar-Neuroendocrine-Ductal Carcinoma with Amphicrine Features and MMR Deficiency

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Introduction:
Mixed acinar-neuroendocrine-ductal carcinoma is exceptionally rare, with only fourteen reported cases. Here, we present a case of this uncommon entity, showcasing intriguing pathologic findings.

Case Presentation:
A 63-year-old male complained of mild abdominal pain, jaundice, nausea, and anorexia. Abdominal computed tomography revealed a dilated common bile duct and a 1.8 x 1.6 cm hypoenhancing mass in the pancreatic head. The cancer antigen 19-9 serum marker was not elevated. An external biopsy diagnosed adenocarcinoma, prompting a pancreaticoduodenectomy.

Pathology:
Gross examination of the pancreaticoduodenectomy specimen uncovered a solid, infiltrating tan-white mass (1.8 x 1.5 x 1.2 cm) centered in the pancreatic head, extending into the ampulla. Microscopic sections revealed a malignant epithelial neoplasm with distinct morphologies. One component displayed well-to-moderately differentiated ductal adenocarcinoma, while the other exhibited areas with solid sheets, nests, and pseudo-glandular patterns. The tumor, located in the pancreatic head, invaded the ampulla, duodenal wall, and peripancreatic soft tissue. Immunohistochemistry (IHC) highlighted CAM 5.2 and CK7 (strongly expressed in the ductal component). The non-ductal component co-expressed trypsin and BCL-10 (acinar markers), as well as synaptophysin, chromogranin A, and INSM-1 (neuroendocrine markers) diffusely, suggesting acinar and neuroendocrine differentiation within the same tumor cells. The Ki-67 proliferation index exceeded 90% in all morphologies. Mismatch repair (MMR) proteins IHC revealed loss of nuclear expression in MSH2 and MSH6 in all tumor components. Three lymph nodes harbored tumor with mixed patterns. Additional findings included a low-grade intraductal papillary mucinous neoplasm (1 cm) and multifocal pancreatic intraepithelial neoplasia, also low-grade.

Conclusions:
The 8th edition of the WHO defines mixed neuroendocrine-non-neuroendocrine (MiNEN) when ≥30% of the neoplasm is composed of each type, and the neuroendocrine component is substantiated by IHC. Our case is unique as it involves a mixed carcinoma with ductal adenocarcinoma and an amphicrine component expressing both acinar and neuroendocrine markers diffusely. An accurate diagnosis is crucial, given that the acinar component is likely to impact prognosis. Furthermore, our case stands out as no previous cases in the literature reported MMR deficiency, prompting consideration of Lynch syndrome in our case, potentially influencing prognosis. Additional studies may enhance understanding of the clinical behavior and prognosis of this entity.

ID# 54

Programmed Cell Death Ligand 1 and Programmed Cell Death 1 in Pediatric Post-Transplant Lymphoproliferative Disorders: Expression Frequency and Genetic Mutations

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Background:
Programmed cell death ligand 1 (PD-L1) is an immunomodulatory molecule overexpressed in lymphomas. In lymphoma with lymphoma cells and the associated tumor microenvironment cells can express PD-L1 (e.g., tumor-associated macrophages (TAMs)) and programmed cell death 1 (PD-1) [e.g., tumor-infiltrating lymphocytes (TILs)], to create the PD1/PD-L1 axis, which is a target for checkpoint inhibitor therapy. PD1/PDL1 expression in both adult and pediatric post-transplant lymphoproliferative disorder (PTLD) is understudied. Here, we investigated PD-1/PD-L1 expression in pediatric PTLD cases, specifically diffuse large B-cell lymphoma (PTLD-DLBCL) and polymorphous PTLD (P-PTLD), which have not been previously studied.

Design:
The cases were retrospectively identified in our archives from 2013-2021. Using immunohistochemistry, PD-L1 (clone E1L3N) expression in tumor and TAMs and PD-1 (clone D4W2J) expression in TILs were evaluated on cases with adequate tissue. Targeted next-generation sequencing using a custom-designed 152 gene panel designed for pediatric hematologic malignancies was performed on 6 PTLD-DLBCL cases with adequate DNA quality.

Results:
The 19 cases included 16 total patients, 12 PTLD-DLBCL patients and 4 P-PTLD patients with a median age of 10 years and 11 years, respectively (Table 1). All cases were EBV positive by EBV in situ hybridization. 93% of the PTLD-DLBCL cases and 83% of the P-PTLD cases, overexpressed tumor PD-L1 while 53% of the PTLD-DLBCL and 30% of the P-PTLD were positive for TAM PD-L1. Both groups received various treatment protocols for the treatment of PTLD. 58% (n=7) of the PTLD-DLBCL cases and 25% (n=1) of the P-PTLD cases are alive without evidence of disease (NED). The median follow-up for all patients was 60 and 35 months, respectively (Table 1). Sequencing of the PTLD-DLBCL cases revealed multiple mutations (2-9 per case), and in 2 of the cases several tier I/II mutations were found involving the CBLB, DDX3X, PCBP1, KRAS, NOTCH1 and SRSF2 genes.

Conclusion:
This study serves to broaden the knowledge of PD-1/PD-L1 expression in pediatric PTLD, and demonstrates PD-L1 overexpression in cases with EBV positivity, similar to that seen in adult PTLD. This may help in directing future therapies to limit the development of PTLD-DLBCL.

ID# 55

The Role of DYRK2 Kinase in Chronic Myeloid Leukemia Stem Cells

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Introduction: Chronic Myeloid Leukemia (CML) originates from hematopoietic stem cells (HSCs) that are transformed by the BCR-ABL1 fusion protein, a product of the t (9;22) (q34; q11) translocation, which possesses constitutive tyrosine kinase activity that drives the proliferation of myeloid cells. Despite treatment with tyrosine kinase inhibitors (TKIs) such as imatinib achieving 10-year survival rates of 82%, 60% of patients experience relapse upon drug discontinuation due to the presence of persistent leukemic stem cells (LSCs). These therapy-resistant LSCs require lifelong TKI use despite the risks of cardiovascular and thromboembolic complications. Our lab recently identified Krüppel-like factor 4 (KLF4) as a regulator of LSC self-renewal and survival in a BCR-ABL1-induced mouse model of CML (Park et al., Blood 2019). Loss of KLF4 inhibited LSC self-renewal and significantly prolonged overall survival. Mechanistically, KLF4 deficient LSCs showed elevated expression of the kinase DYRK2 involved in p53-activation, apoptosis, and c-MYC depletion by the proteasome inhibiting LSC self-renewal. We hypothesize that stabilizing DYRK2 protein expression using genetic or pharmacological strategies will eliminate LSCs, potentially overcoming TKI resistance and enabling lasting treatment-free remission. This work aims to validate DYRK2 as a therapeutic target and identify small molecules that exploit its tumor-suppressive functions to eradicate CML LSCs.

Methods: We generated a mouse CML model by transducing hematopoietic stem/progenitor cells (HSPCs) with retroviruses expressing *BCR-ABL1* and *DYRK2*. HSPCs (Lin^- $Sca1^+$ $cKit^+$) from C57BL/6 mice were co-transduced with *BCR-ABL1-IRES-RFP* and *empty-IRES-GFP* retroviruses (control), and *BCR-ABL1-IRES-RFP* and *DYRK2-IRES-GFP* retrovirus (experimental). GFP^+ RFP^+ cells were purified by cell sorting and injected into lethally irradiated (950 Rad) mice with radioprotective cells. Mice were monitored in peripheral blood by flow cytometric detection of leukemic cells (GFP^+ RFP^+). Bone marrow/spleen leukemic cells were analyzed post-mortem for c-MYC, p53, and DYRK2 expression by immunoblots. Using established human CML cell lines, we screened the effect of drugs (ubiquitin ligase, KLF4, and proteasome inhibitors) on cell viability and expression of DYRK2 and downstream target c-Myc.

Results: In the mouse model, we observed lower expansion of GFP^+(DYRK2) RFP (BCRABL1) $^+$ leukemic cells in peripheral blood, suggesting that DYRK2 expression alone is enough to inhibit the expansion of CML cells. This is consistent with an inhibition of colony-forming assays in methylcellulose cultures. The whole blood counts reveal a lower expansion of neutrophils by ectopic DYRK2 expression in CML cells. In the K562 and KCL22 cell lines, we found dose-dependent cytotoxicity with betulinic acid (11.23 μM), adapalene (12.68 μM), MG132 (238 nM), SP141 (148 nM), kenpaullone (4.3μM), and imatinib (171 nM), which was linked to the upregulation of DYRK2 and depletion of c-Myc.

Conclusions: Our research shows that DYRK2 alone inhibits CML progression by activating p53-mediated apoptosis and reducing LSC self-renewal through c-Myc depletion. These findings indicate that DYRK2 functions as a tumor suppressor in CML by limiting LSC maintenance and promoting myeloid differentiation. As future directions, we will perform proteomic analysis to identify additional targets of DYRK2 in CML cells, requirement of kinase activity, establish the role of the ubiquitin ligase SIAH2 in DYRK2 proteolysis, and screen for small molecules able to stabilize DYRK2 protein. This project will aid in developing LSC-specific drug therapy to achieve treatment-free remission in CML patients.

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Assessing the Antileukemic Potential of Neratinib in T-ALL

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Introduction. Leukemia is the most prevalent pediatric cancer, accounting for 31% of all childhood cancer cases, according to SEER data. Among these, acute lymphoblastic leukemia (ALL) constitutes the majority, representing 26% of all cases in children under 15 years, with peak incidence at ages 3–5. Over the past decades, advancements in ALL treatment have increased survival rates from 21% in the 1960s to over 80-85% today. However, patients with relapsed leukemia face significantly worse outcomes, with a two-year survival rate of only 40%. For T-cell acute lymphoblastic leukemia (T-ALL), the five-year survival rate is 85.5%, but relapsed or refractory T-ALL has a dismal prognosis. This poor outlook for relapsed/refractory T-ALL underscores the urgent need for novel or repurposed therapeutic strategies. Genomic studies have identified the MAPK signaling pathway as a critical and potentially targetable pathway in pediatric leukemia. Our lab has discovered aberrant activation of MAP2K7 in pediatric T-ALL. MAP2K7 exclusively activates JNK, a kinase involved in cell viability and proliferation. JNK inhibition has shown significant anti-leukemic effects, including reduced leukemia burden in mouse models. Furthermore, MAP2K7 knockout in T-ALL cell lines abrogated JNK phosphorylation, inhibited cell growth, increased apoptosis, and improved survival *in vivo*, supporting MAP2K7 as a therapeutic target for pharmacological inhibition.

Neratinib is an irreversible pan-HER tyrosine kinase inhibitor primarily targeting HER2 and EGFR signaling pathways. It is FDA-approved for the extended adjuvant treatment of HER2-positive breast cancer following trastuzumab therapy. By covalently binding to cysteine residues in the ATP-binding pocket of HER family receptors, neratinib prevents receptor autophosphorylation and downstream oncogenic signaling, suppressing tumor growth and proliferation. We hypothesize that neratinib, in combination with standard-of-care chemotherapies, will enhance treatment efficacy by inhibiting MAP2K7-driven JNK signaling, reducing leukemia cell survival, and promoting apoptosis.

Methods. Cell viability assays (CellTiter-Glo) to evaluate the efficacy of neratinib across a panel of T-ALL cell lines and determine IC50 values. In-vitro kinase assays using recombinant MAP2K7 and its substrate JNK to assess the ability of neratinib to inhibit MAP2K7 kinase activity directly. Immunoblot analysis is used to validate MAP2K7 inhibition by determining downstream targets such as JNK and ATF-2 phosphorylation levels. Flow cytometry analysis to determine apoptosis using Annexin V staining and cell cycle analysis by nuclei staining with propidium iodine.

Data/Conclusion. The kinase assay shows that neratinib can directly inhibit MAP2K7 activity and is highly cytotoxic against multiple T-ALL cell lines. Western Blot data strongly indicates that neratinib effectively inhibits MAP2K7 activity, as evidenced by the absence of phosphorylated JNK and reduced levels of phosphorylated ATF-2 in sorbitol-induced KOPT-K1 cells. These findings suggest that neratinib disrupts MAP2K7-mediated activation of the JNK signaling pathway, reinforcing its potential as a therapeutic strategy for T-ALL.