immune-suppressive changes in surrounding E cells, which protects them against immune attack. Finally, I found that canine mammary tumors with higher proportions of qM tumor cells assemble an immune-suppressive tumor microenvironment, highlighting the translational potential of our findings. DISCUSSION/SIGNIFICANCE OF IMPACT: We identified that the epithelial–mesenchymal transition induces immune-suppressive changes in heterogeneous tumors. These findings may reveal novel therapeutic targets for treatment of refractory tumors. Our findings in canine tumors suggest that these mechanisms are conserved across species.

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Buffering the impact of violence exposure: The role of caregiver and peer support on adolescent brain connectivity*

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OBJECTIVES/GOALS: Adolescence is a critical period where brain networks are thought to be influenced by environmental factors. This presentation examines violence exposure's impact on brain connectivity and identifies potential protective factors. METHODS/STUDY POPULATION: A secondary data analysis was conducted using data from a subsample of the Adolescent Brain Cognitive Development Study (release 5.1). Youth who completed victimization questionnaires at two time points were eligible for inclusion, resulting in 2016 participants. Linear regression was utilized to analyze associations between violence exposure measured by the juvenile victimization questionnaire and functional connectivity of specified regions of interests using the Gordon functional parcellation for cortical regions and the Freesurfer parcellation for subcortical regions. Moderation analysis will be utilized to assess the effects of peer and caregiver support on the associations between violence exposure and functional connectivity, currently ongoing. RESULTS/ANTICIPATED RESULTS: Between 18 and 59% of the sample reported experiencing at least one form of violence exposure, with racial differences noted in missing versus complete data. Multiple domains of violence and cumulative exposure were associated with both increased and decreased functional connectivity across within-network, between-network, and network-subcortical regions. At baseline, internet violence was linked to lower within-network connectivity, while peer victimization was associated with higher connectivity at both baseline and follow-up. Between network analysis showed lower connectivity with witnessing violence at baseline and higher connectivity with internet victimization at follow-up. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings emphasize the need for further exploration of the underlying mechanisms that link violence exposure to developmental trajectories and identification of protective factors such as caregiver and peer support, to inform interventions and promote resilience in affected youth.

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The diffusion of vaginal bacterial extracellular vesicles through cervicovaginal mucus facilitates inflammation in female reproductive tract cells

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OBJECTIVES/GOALS: To probe microbe and bacterial extracellular vesicle (bEV) mobility through biological barriers, we use novel multiple-particle tracking technology. The goal is to evaluate changes caused by extracellular vesicles relevant to placental function and neonatal development. METHODS/STUDY POPULATION: We conducted multiple particle tracking to assess whole bacterial and bEV mobility in cervicovaginal mucus. To accomplish this, cervicovaginal mucus was self-collected from 10 women. Mucus samples were characterized via wet mount, Nugent score, and pH measurements. In parallel, we cultured commercially available vaginal bacteria strains in anaerobic conditions. We isolated bEVs via ultracentrifugation, and subsequently characterized them via nanoparticle tracking analysis to measure size, ζ-potential, and concentration. We investigated reproductive tract tissues response to bEVs. We dosed vaginal, endometrial, myometrial, and placental cells lines with bEVs over a 24 h period and determined uptake, viability, and cytokine production. One-way analysis of variance was used for statistical analysis. RESULTS/ANTICIPATED RESULTS: Based on our previous work, size and ζ-potential greatly affect particle mobility in mucus. *G. vaginalis* and *M. mulieris* were smaller than *L. crispatus* and *L. iners*. *G. vaginalis* had a more net-neutral ζ-potential compared to other bEVs. During multiple-particle tracking analysis, whole bacteria were unable to diffuse through vaginal mucus, while bEVs showed increased mobility. Through fluorescence levels, we determined *M. mulieris* bEVs reach >90% uptake at 24 h. Uptake was verified via microscopy. Across all strains, bEVs were not detrimental to placental viability. When investigating cytokine production in placental cells, an increase in IL-6 was seen after treatment with *L. iners* bEVs, while TNFα was increased after treatment with *G. vaginalis* bEVs. DISCUSSION/SIGNIFICANCE OF IMPACT: Vaginal microbiome dysbiosis increases adverse obstetric indications. We demonstrate that bacteria are unable to ascend to reproductive tissues. We propose that bEVs travel through vaginal mucus, facilitating microbe–host communication. This impacts obstetric disease pathology and is relevant for diagnostic criteria during pregnancy.

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CD8+ T-cell transfer induces adverse alterations to the post-myocardial infarction scar*

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OBJECTIVES/GOALS: Cardiovascular disease, particularly myocardial infarction (MI), is a leading cause of death in the USA. Previous studies have identified CD8+ T-cells as adverse regulators post-MI. We hypothesized that CD8+ T-cells impair cardiac function by altering scar composition. METHODS/STUDY POPULATION: MI was

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induced by permanent ligation of the coronary artery in C57BL6/J (WT; 3–7 mo) and CD8atm1mak mice (CD8-/-; 3–7 mo). CD8-/- mice were injected with either vehicle or naïve splenic CD8+ T-cells (2x10^6 cells/injection) via tail vein, 4 hours after ligation. Tissue was collected at Day 7 post-MI for biomechanical testing and further downstream analyses. Granzyme (Gzm)A, B, and K were tested for collagen cleavage using a fluorogenic cleavage assay. Effect on collagen production in TGF-β-activated cardiac fibroblasts was assessed in vitro by stimulating cells with GzmA, B, and K (25 AU) for 24 hours. RESULTS/ANTICIPATED RESULTS: CD8-/- mice had improved ejection fraction and LV dilation at Day 7 post-MI compared to WT and CD8-/- mice resupplemented with splenic CD8+ T-cells (p DISCUSSION/SIGNIFICANCE OF IMPACT: Our study demonstrates that CD8+ T-cells regulate cardiac fibrosis partially through Gzm release, resulting in left ventricular biomechanical impairments and increased dilation.

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The MEK1/2 inhibitor ATR-002 has dual anti-inflammatory and antibacterial effects during *S. aureus* infection*

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OBJECTIVES/GOALS: Novel therapeutics to control *Staphylococcus aureus* (*S. aureus*) infections are needed for people with cystic fibrosis (CF, PwCF). In this study, our objective is to determine if the pharmacologic MEK1/2 inhibitor compound ATR-002 can restrict the growth of *S. aureus* clinical isolates and modulate infection in a murine model of *S. aureus* infection. METHODS/STUDY POPULATION: To evaluate the anti-inflammatory effects of ATR-002 on human macrophages, cells were stimulated with TLR2 agonists FSL1 or Pam3CSK4 with a dose range of ATR-002, and secretion of proinflammatory cytokines were measured by ELISA. To determine the direct antibacterial effect of ATR-002, minimum inhibitory concentration (MIC) assays were performed with the community-associated methicillin-resistant *S. aureus* strain USA300 and 40 *S. aureus* isolates from PwCF. To validate our results in vivo, mice were provided i.p. treatment with either vehicle, the MEK1/2 inhibitor compound PD0325901 (20 mg/kg), or ATR-002 (10 mg/kg) prior to intranasal infection with 1x10^7 CFU of USA300. Bacterial burdens at 4- and 24-hour post-infection (p.i.) and inflammatory cell recruitment at 24 hours p.i. were quantified. RESULTS/ANTICIPATED RESULTS: Macrophages treated with ATR-002 exhibited a dose-dependent decrease in secretion of proinflammatory cytokines TNF and IL-8 following TLR stimulation. Our studies identified that ATR-002, but not PD0325901 or other MEK1/2 inhibitors, had direct antibacterial effects, and ATR-002 had an MIC range of 8 to above 64 ug/mL on CF *S. aureus* isolates. In the murine pulmonary infection model, delivery of ATR-002 and PD0325901 significantly prevented infection-induced loss of body mass and decreased neutrophil inflammation. However, when bacterial burdens were quantified 4-hours p.i., only ATR-002 treatment reduced lung bacterial burden compared to vehicle or PD0325901-treated groups. DISCUSSION/SIGNIFICANCE OF IMPACT: These results are the first demonstration of the in vivo anti-inflammatory and antibacterial effects of ATR-002. Our results further demonstrate that ATR-002 exhibits direct antibacterial effects across a collection of clinical isolates of *S. aureus*. Future studies will continue to investigate the therapeutic potential of ATR-002.

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Harmonization of quality indicators in Clinical Microbiology Laboratories

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OBJECTIVES/GOALS: This study aims to harmonize quality indicators (QIs) across University of Toronto-affiliated microbiology labs to establish universal benchmarks that enhance performance, patient safety, and health outcomes. Harmonized QIs will enable effective comparisons and enhance the consistency of care. METHODS/STUDY POPULATION: The study employed the Delphi method, a structured and iterative process to build consensus. An expert panel of clinical microbiology trainees, medical microbiologists, trainees, and site leads from five University of Toronto-affiliated microbiology labs was assembled. Initial insights were gathered through surveys and a comprehensive scoping review of the literature. The study involved two rounds of feedback, a SurveyMonkey-based survey, with a defined consensus of 75% agreement among participants. Followed by an implementation survey conducted through REDCap to assess how these QIs were adopted in practice and identify barriers to implementation. RESULTS/ANTICIPATED RESULTS: The study achieved consensus on nine high-impact quality indicators, including blood culture volume and contamination rates, cerebrospinal fluid transport time, and turnaround times for Gram stain results. Blood culture contamination and positivity rates garnered the highest agreement, at 100% and 91%, respectively. While some indicators were widely accepted and implemented, others faced resistance due to feasibility concerns. The study also identified significant variability in the level of adoption across the participating laboratories, pointing to operational challenges and the need for further efforts to address these barriers. DISCUSSION/SIGNIFICANCE OF IMPACT: This study highlights the importance of QI harmonization in improving lab services and patient safety. It reveals challenges in standardizing practices but promotes uniformity in QIs, laying the groundwork for better inter-lab collaboration, consistent outcomes, and improvements in microbiology.

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Preclinical drug screen leads to clinical trial for treatment of hypoglycemia unawareness in type 1 diabetes mellitus*

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OBJECTIVES/GOALS: People with insulin-treated diabetes face hypoglycemia risk due to imperfect insulin replacement and impaired counterregulation. We identified the dopamine antagonist, metoclopramide, as a potential treatment. Hypothesis: Treatment with metoclopramide will prevent the development of impaired counterregulatory response to hypoglycemia. METHODS/STUDY POPULATION: In a pre-clinical model, diabetes was induced in 10-week-old Sprague-Dawley rats with streptozotocin (STZ, 65 mg/kg IP). Rats were divided into three groups: 1) diabetic controls

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