

POPULATION: Washed platelets or platelet rich plasma from healthy human donors were treated with EPA and 12-HEPE to assess their ability to inhibit platelet activation. Platelets were stimulated with agonists targeting different steps of the hemostatic response to vascular injury. Platelet aggregation, dense granule secretion, surface expression of integrin  $\alpha\text{IIb}\beta\text{3}$  and P-selectin, and clot retraction were analyzed. To assess signaling through G $\alpha$ s-GPCRs and protein kinase A activity, phosphorylation of vasodilator-stimulated phosphoprotein (VASP) was examined via western blot following treatment with EPA or 12-HEPE. RESULTS/ANTICIPATED RESULTS: EPA and 12-HEPE dose-dependently inhibit both collagen and thrombin-induced platelet aggregation. Furthermore, 12-HEPE more potently attenuates dense granule secretion and surface expression of platelet activation markers, integrin  $\alpha\text{IIb}\beta\text{3}$  and P-selectin, in comparison to EPA. Plasma treated with EPA delayed thrombin-induced clot retraction, while 12-HEPE had no effect. Additionally, treatment with 12-HEPE increases phosphorylation of VASP, suggesting it could signal through the activation of the eicosanoid G $\alpha$ s-GPCRs. DISCUSSION/SIGNIFICANCE: Here, we show for the first time that EPA directly inhibits platelet activation through its 12-LOX metabolite, 12-HEPE. These findings provide further insight into the mechanisms underlying the cardioprotective effects of EPA. A better understanding of current PUFA supplementations can inform treatment and prevention of cardiovascular diseases.

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### Intramuscular immunization with rVCG-MECA vaccine elicits stronger chlamydial specific immune response than intranasal immunization

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OBJECTIVES/GOALS: We prioritize Chlamydia's public health impact, aim to develop rVCG-MECA for practical use, study robust immunity for effective strategies, and assess animal models for human vaccination adaptation. Our work highlights rVCG-MECA's translational significance in public health. METHODS/STUDY POPULATION: Female Mice C57BL/6J mice (N=8) were immunized intramuscularly(IM) and intranasally(IN) and boosted twice, two weeks apart, with rVCG-MECA, once with live Chlamydia (*C. trachomatis* serovar D elementary bodies) and PBS. Specific mucosal and systemic immune responses were characterized. Vaccine efficacy was determined from chlamydia shedding following the transcervical challenge. Additionally, Chlamydia-specific cytokine (IFN- $\gamma$  and IL-4) production by splenic and ILN T cells was assessed after 16 weeks RESULTS/ANTICIPATED RESULTS: Immunization with rVCG-MECA via intramuscular and intranasal routes triggered notable humoral responses in systemic and mucosal tissues. Intramuscular vaccination produced higher IgG2c levels in both tissues, while intranasal vaccination led to elevated IgA levels in mucosal tissues. rVCG-MECA-immunized mice exhibited significantly higher IFN- $\gamma$  (Th1) secretion compared to IL-4 (Th2), with intramuscular immunization showing the highest IFN- $\gamma$  levels. These findings anticipate robust immune responses, promising protection against Chlamydia, particularly through the intra muscular route. Overall, our results support rVCG-MECA as a promising Chlamydia vaccine, aligned with public health goals. DISCUSSION/SIGNIFICANCE: This study

suggests that IM and IN immunization with rVCG-MECA induces immune effectors such as IFN-gamma and IgG2c that mediate chlamydial clearance in the genital tract.

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### Computable Phenotyping with "Big Data" as a Foundation for Artificial Intelligence Algorithm Construction: Puberty as a Transdisciplinary Case Example

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OBJECTIVES/GOALS: Artificial intelligence (AI) depends on quality machine learning (ML) algorithms constructed with high-quality training data. This TL1 trainee project develops a disease-agnostic computable phenotype framework for ML algorithm construction, modeling male puberty as a case example. METHODS/STUDY POPULATION: A computable phenotype of male puberty was constructed to answer the question: "Does early pubertal timing increase the risk of developing type II diabetes (T2D) in males?" A computable phenotype of males < 18 years old was created in the TriNetX<sup>©</sup> Diamond Network utilizing Boolean operator data queries. TriNetX<sup>©</sup> contains patient electronic health record information (ICD-10 diagnoses, anthropometric measures). An exploratory analysis of patient counts reflecting various computable phenotypes allowed for outcome (T2D) comparison of males diagnosed with precocious puberty (E30.1, ICD code for early pubertal timing) to those without, controlling for body mass index (BMI). RESULTS/ANTICIPATED RESULTS: Subjects (n=12,996,132) displayed the following computable phenotype: Male, < 18 years old, without ever having a BMI documented >85th percentile. Males diagnosed with precocious puberty (E30.1) were 6.89 times more likely to develop T2D when aged 14-18 years old than those without (OR 6.89, 95% CI: 5.17-9.19, p<0.0001). Next steps involve training a ML model on each computable phenotype groupings' health data, with anticipated results identifying underlying salient pathophysiologic variables. A generalized computable phenotype approach is further developed to: 1) explore clinical questions in large databases like TriNetX<sup>©</sup>, and 2) model disease development with AI/ML algorithm construction. DISCUSSION/SIGNIFICANCE: Computed phenotypes reveal males with precocious puberty may have increased T2D risk. Next steps utilize subject data to train an AI/ML algorithm, model development to identify salient pathophysiologic variables, and synthesize a generalized AI/ML developmental research framework for dissemination.

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### Innovation in MS Patient Care: Linking Cognitive Health and Myelin Integrity

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OBJECTIVES/GOALS: Our objective is to develop a patient-friendly application addressing the progression of cognitive impairments in multiple sclerosis (MS) patients. This initiative aims to augment individualized care and precision management of a major MS comorbidity by generating a cognitive health brain map for each patient. METHODS/STUDY POPULATION: Using the UAMS COMS

Database, featuring high-resolution multi-contrast MRIs, and a comprehensive clinical, behavioral, and demographic dataset, we are developing a hierarchical learning-based software tool to compute maps correlating brain structure-function and individual cognitive function. Our MRI analysis employs a three-compartment model (NNLS>0.96). Functional scores are defined by individualized accuracy during the modified information processing speed task (e.g., m-SDMT). We utilize a Bayesian classifier with explicit Pearson's correlation for tissue classification (BF10>100) to compute an index of the likelihood of correlation with cognitive impairment throughout brain tissue. RESULTS/ANTICIPATED RESULTS: This approach allows us to reveal subtle cognitive changes and their potential links to myelin integrity, offering vital insights into disease progression and management. The m-SDMT strongly correlates with the standard SDMT ( $r=0.79$ ,  $p<0.001$ ), confirming reliability as a cognitive assessment tool in clinical and research contexts. Analysis of the COMS dataset emphasized insights into the role of fine myelin structure in MS patients' cognitive functionality. Our findings heightened the pivotal significance of myelin integrity in preserving cognitive abilities and identify disruptions in myelin synthesis and homeostasis as primary contributors to cognitive decline. This discovery stresses the critical role that specialized brain pathways, influenced by myelin integrity, play in the pathology of MS. DISCUSSION/SIGNIFICANCE: This development bridges advanced neuroimaging techniques with practical clinical applications, emphasizing the nuanced role of myelin integrity in MS-related cognitive deficits. Our findings advocate for a multidisciplinary approach to MS management, demanding collaborative workforce development and education in translational science.

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### Deciphering the Immune Landscape in Benign Breast Disease: Implications for Risk Stratification and Breast Cancer Prevention

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OBJECTIVES/GOALS: The objective of our research is to define unique molecular and immune markers in benign breast tissue to better identify women at risk of node-positive breast cancer (BC). The goal of the work is to improve individualized risk assessment, to guide targeted prevention and screening recommendations, and to reduce disease incidence and mortality. METHODS/STUDY POPULATION: From the Mayo Clinic's Benign Breast Disease (BBD) cohort, we matched women who developed node-positive breast cancer after a BBD biopsy (cases;  $n=42$ ) with women who remained cancer-free (controls;  $n=37$ ), considering patient age and biopsy date. We used NanoString gene expression profiling to identify differentially expressed genes (DEGs) between cases and controls. We optimized a multiplex immunofluorescence (mIF) approach to simultaneously detect multiple markers within single FFPE tissue slides to correlate cells expressing DEGs in relation to innate and adaptive immune effectors. We used tissue segmentation, cell phenotyping, and spatial relationships to define molecular and immune differences between cases and controls. RESULTS/ANTICIPATED RESULTS: We discovered higher gene expression levels of IRF8 (interferon regulatory factor 8, a factor involved in immune cell differentiation) in controls as compared to cases

( $p = 0.0024$ ) and found that IRF8 expression is also associated with longer cancer onset times among cases ( $p = 0.0012$ ). Our pilot mIF experiments revealed higher frequencies of CD4+, CD8+, CD68+, CD20+ and CD11c+ cells in controls compared to cases. We predict that higher IRF8 expression and increased frequencies of immune cells in BBD biopsies indicate a proactive immune environment that may act to prevent cancer development. Furthermore, we predict that our analyses of the spatial localization of these markers by mIF may offer further predictive insight. DISCUSSION/SIGNIFICANCE: Deciphering the relationship between immune alterations in BBD and risk of node positive BC has the potential to improve individualized risk prediction. These insights will foster improved surveillance and informed screening and prevention, ultimately reducing BC incidence and mortality.

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### Anifrolumab for the treatment of refractory cutaneous lupus erythematosus in patients: interim analysis of real-world outcomes

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OBJECTIVES/GOALS: \* Patients with skin of color (SOC) are disproportionately affected by systemic lupus erythematosus (SLE) and cutaneous lupus erythematosus (CLE). In this study, we aim to address this disparity and characterize the real-world efficacy and tolerability of anifrolumab in CLE patients using validated disease activity instruments. METHODS/STUDY POPULATION: This single-center, prospective observational cohort study includes SLE patients with severe or refractory CLE who have received  $\geq 1$  dose of anifrolumab. Cutaneous disease activity is assessed periodically at 2, 6, 9, 12, and 18 months using the Cutaneous Lupus Disease Area and Severity Index (CLASI). Adverse events and concurrent treatments are also routinely evaluated. To date, 22 patients have been enrolled, with 6-month follow-up data available for 15. At the time of anifrolumab initiation, 95% of participants had discoid LE (DLE), 60% had mucosal DLE, and 13% had subacute CLE. Nine patients identified as SOC, two as White, and four did not report race/ethnicity. RESULTS/ANTICIPATED RESULTS: A Friedman test showed statistically significant changes over time in CLASI activity score (CLASI-A) ( $\chi^2(2) = 20$ ,  $p<0.0001$ ) (Figure 1) and CLASI damage score (CLASI-D) ( $\chi^2(2) = 9.5789$ ,  $p=0.0083$ ) (Figure). To estimate effect sizes, we employed linear mixed models, which demonstrated statistically significant reductions in the CLASI-A score from baseline by an average of 14 points at 2 months ( $p<0.001$ ) and 18 points at 6 months ( $p<0.001$ ); notably, a reduction in CLASI-A of 4 is considered clinically meaningful. At 2 months, 20% of patients experienced a 50% or more reduction in CLASI, which increased to 60% of patients at 6 months. Patients on systemic corticosteroids could taper off. Adverse events were minimal and did not lead to treatment discontinuation. Fig. 1:[blob:https://acts.slayte.com/045319b4-7272-4351-a771-78ba9ee57f5c] Fig. 2:[blob:https://acts.slayte.com/