RARRES1-depleted epithelial cells. Extracellular flux assays were used to assess the phenotype of RARRES1-depleted epithelial cells treated with or without metformin. RESULTS/ANTICIPATED RESULTS: RARRES1 is a major regulator of mitochondrial function. Its depletion in tumors induces an oxidative phosphorylation dependent phenotype and subsequently increases ATP abundance in the cell, enhances anabolic pathways and increases survival. Treatment with FDA approved mitochondrial respiration inhibitor, metformin, reversed the metabolic phenotype of RARRES1 depleted-epithelial cells. Metformin could be the ideal therapeutics to reduce tumor burden in cancers with loss of or low expression of RARRES1. DISCUSSION/SIGNIFICANCE OF IMPACT: Bioenergetic dynamics are emerging as a basis for understanding the pathology of cancer. The malignancy progresses as its metabolic pattern and mitochondrial respiration become more dysfunctional. The regulatory pathways of bioenergetic dynamics are currently poorly understood, and the characterization of proteins implicated in those processes must be assessed. One understudied protein and tumor suppressor is RARRES1. RARRES1 is induced by retinoic acid (a major metabolic regulator) and functions as a putative carboxypeptidase inhibitor. Understanding the connection between this carboxypeptidase inhibitor and intermediary metabolism will enlighten our understanding of the bioenergetic profile of cells and facilitate the discovery of personalized metabo-therapeutics in the context of cancer.

**2436**

## Disagreement in middle ear volume values between tympanometry and 3-dimensional volume reconstruction

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OBJECTIVES/SPECIFIC AIMS: Middle ear volume (MEV) is a clinically relevant parameter in the treatment of many common conditions including otitis media, tinnitus, and conductive hearing loss. A growing number of studies have shifted from using tympanometry to 3-dimensional volume reconstruction (3DVR) to calculate MEV; however, MEV values between these methodologies have never before been directly compared. Here, our objective is to characterize agreement between MEV measurement methods across disease states and middle ear sizes. METHODS/STUDY POPULATION: Middle ears were identified from 36 patients ranging 18–89 years of age who underwent tympanometry testing during preoperative workup for tympanic membrane (TM) perforation, up to 1 month prior to a standard-of-care temporal bone computed tomography (CT) between October 15, 2005 and October 15, 2015. MEV values calculated by both tympanometry and 3DVR were analyzed for agreement using Bland and Altman plots. A correction factor was calculated where ear canal volumes were available for contralateral middle ears without TM perforation (n = 12), and was applied to a second Bland and Altman plot in the corresponding patient subgroup. MEV agreement was characterized across MEV quartiles (1 = smallest; 4 = largest) and across increasing states of middle ear disease using Kruskal-Wallis and Wilcoxon testing with Bonferonni correction. RESULTS/ANTICIPATED RESULTS: A Bland Altman plot demonstrated significant disagreement of MEV differences as compared to a priori clinical thresholds. Absolute MEV difference was significantly greater in the average MEV fourth to first quartile ($p = 0.0024$), fourth to second quartile ($p = 0.0024$), third to first quartile ($p = 0.0048$), and third to second quartile ($p = 0.048$). Absolute MEV difference was not significantly different across varying states of middle ear disease ($p = 0.44$). DISCUSSION/SIGNIFICANCE OF IMPACT: Statistically evident and clinically significant disagreement was demonstrated across tympanometric and 3DVR MEV estimates. This lack of agreement was most pronounced at higher average MEV and was persistent yet not appreciably different across varying severities of middle ear disease. These findings may limit the generalizability of studies of the middle ear that differ in MEV estimation methodology, particularly in pathophysiological states where MEV is increased.

**2504**

## Defining peripheral B cell tolerance in pemphigus vulgaris

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OBJECTIVES/SPECIFIC AIMS: Pemphigus vulgaris (PV) is a potentially fatal blistering disease caused by autoantibodies to the keratinocyte adhesion protein desmoglein 3. Several other autoimmune diseases have defective B cell tolerance checkpoints, resulting in the accumulation of self-reactive and polyreactive B cells. METHODS/STUDY POPULATION: The present work aims to determine whether PV patients develop normal tolerance to self-antigens other than desmoglein 3, as a potential "first hit" in the development of autoimmunity. We use FACS to isolate single B cells at 4 developmental stages from 8 PV patients. We perform single-cell RT-PCR to amplify each B cell receptor, produce monoclonal antibodies, and screen these for autoreactivity using ELISA/IF to several self-antigens. At each B cell stage, we compare the frequencies of self-reactive and polyreactive B cells to those found in healthy controls. RESULTS/ANTICIPATED RESULTS: We anticipate similar frequencies between PV patients and controls, suggesting that the B cell repertoire in PV patients develops normally at early checkpoints. DISCUSSION/SIGNIFICANCE OF IMPACT: The absence of generalized reactivity would distinguish PV from other autoimmune diseases and would show that PV arises from a specific break in tolerance to a single self-antigen (desmoglein 3) during late B cell maturation. Such a result would further support PV as an ideal candidate for targeted immunotherapy.

**2508**

## The role of platelet factor-4 (PF4 or CXCL4) in B cell differentiation

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OBJECTIVES/SPECIFIC AIMS: To investigate the role of platelet factor-4 (PF4) in B cell differentiation and develop strategies to better modulate B cell differentiation in vitro and in vivo. METHODS/STUDY POPULATION: We use tissue culture and flow cytometry to examine the role of PF4 in B cell differentiation. We use wild type (WT) and $PF4^{-/-}$ mice on a C57Bl6/J background. $PF4^{-/-}$ mice have reduced in vivo B cell differentiation responses. RESULTS/ANTICIPATED RESULTS: We anticipate that our studies will demonstrate that PF4 promotes B cell differentiation in the bone marrow microenvironment. DISCUSSION/SIGNIFICANCE OF IMPACT: The significance of this project may be valuable in developing efficient methods and strategies to increase or limit B cell numbers in vitro and in human disease.

**2509**

## Estimation of HIV viral load using quantitative measurement of HIV-p24 on lateral flow immunoassays

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OBJECTIVES/SPECIFIC AIMS: High-sensitivity diagnostics for early infant diagnosis (EID) of HIV at the point of care (POC) are not widely available. Lateral flow immunoassays (LFA) can detect HIV-p24, but are not sensitive enough in practice. With improvements, LFA are a compelling platform for POC in EID. We used functionalized magnetic beads and immunocomplex dissociation to improve sensitivity of HIV-p24 LFA. Here, we evaluate the utility for LFA to quantitatively report HIV-p24 concentration and estimate HIV viral load. Using purified p24 protein and virion constructs, we determined the limits of detection for HIV-p24 using LFA rapid tests. Using measurements from HIV-p24 ELISA, laboratory-developed RT-qPCR, droplet digital PCR, and gold standard clinical viral load, we further characterized the relationship between HIV-p24 concentration, HIV genomic RNA, and LFA test line signal. METHODS/STUDY POPULATION: We measured HIV-p24 concentration by ELISA (R&D Systems) and LFA (Alere Determine HIV-1/2 Ab/Ag Combo). An LFA reader instrument was used to image test lines and measure test line signal on the LFA. HIV viral loads were measured using RT-qPCR and droplet digital RT-PCR protocols adapted in our lab. We obtained gold standard viral load measurements using the Roche Cobas TaqMan system at Vanderbilt University Medical Center. Data analysis was performed using Prism 7 and Stata 14. RESULTS/ANTICIPATED RESULTS: LFA test line signal increases in a predictable, dose-dependent manner and correlates with concentration of purified HIV-p24 with a linear range between 50 and 1000 pg/mL (Spearman $r = 1$; $p = 0.0004$). We compared p24 concentration (ELISA). We evaluated the utility of LFA to quantify HIV-p24 from virions suspended in human plasma, which increased the limit of detection for HIV-p24 to 100 pg/mL and shifted the linear range 100–10,000 pg/mL (Spearman $r = 0.77$; $p < 0.001$). To evaluate the relationship between HIV-p24 concentration and concentration of HIV RNA, we employed 3 molecular techniques. The LFA is capable of detecting HIV-p24 concentrations that correspond to a range of viral loads between 653,000 and

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