

therapeutic intervention for the prevention of impaired hypoglycemic counter-regulation in persons with diabetes.

4400

### Low CD4 nadir linked to widespread cortical thinning in adults with HIV

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**OBJECTIVES/GOALS:** The history of immune suppression, especially CD4 nadir, has been shown to be a strong predictor of HIV-associated neurocognitive disorders (HAND). However, the potential mechanism of this association is not well understood. This study examined the relationship between CD4 nadir and brain atrophy. **METHODS/STUDY POPULATION:** Fifty-nine people with HIV participated in the cross-sectional study (mean age, 56.5±5.8; age range, 41-69; 15 females; 46 African-Americans). High resolution structural MRI images were obtained using a 3T Siemens scanner. From a comprehensive 7-domain neuropsychological test battery, a global deficit score (GDS) and HAND diagnoses were determined for each participant. The correlation between CD4 nadir (the lowest ever lymphocyte CD4 count) and cortical thickness was investigated using a vertex-wise non-parametric approach with a conservative statistical threshold of  $p < 0.05$  (FWE-corrected). **RESULTS/ANTICIPATED RESULTS:** Out of the 59 participants, 12 met standard Frascati criteria for asymptomatic neurocognitive impairment (ANI) and two met the criteria for mild neurocognitive disorder (MND). Across all participants, low CD4 nadir was associated with widespread cortical thinning, especially in the frontal and temporal regions. Higher GDS (indicating worse global neurocognitive function) was associated with bilateral frontal cortical thinning, and the association largely persisted in the subset of participants who did not meet HAND criteria. **DISCUSSION/SIGNIFICANCE OF IMPACT:** These results suggest that the low CD4 nadir may be associated with widespread neural injury in the brain, especially in the frontal and temporal regions. This spatial profile might contribute to the prevalence/phenotypes of HAND in the cART era, such as the frequently observed deficits in the executive domain.

4009

### Magneto-electric nanoparticles (MENs) cobalt ferrite-barium titanate (CoFe<sub>2</sub>O<sub>4</sub>-BaTiO<sub>3</sub>) for non-invasive neuromodulation<sup>†</sup>

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**OBJECTIVES/GOALS:** Our goal is to develop a non-invasive stimulation technique using magneto-electric nanoparticles (MENs) for inducing and enhancing neuronal activity with high spatial and temporal resolutions and minimal toxicity, which can potentially be used as a more effective approach to brain stimulation. **METHODS/STUDY POPULATION:** MENs compose of core-shell structures that are attracted to strong external magnetic field (~5000 Gauss) but produces electric currents with weaker magnetic field (~450 Gauss). MENs were IV treated into mice and drawn to the brain cortex with a strong magnetic field. We then stimulate MENs with a

weaker magnetic field via electro magnet. With two photon calcium imaging, we investigated both the temporal and spatial effects of MENs on neuronal activity both *in vivo* and *in vitro*. We performed mesoscopic whole brain calcium imaging on awake animal to assess the MENs effects. Furthermore, we investigated the temporal profile of MENs in the vasculatures post-treatment and its toxicities to CNS. **RESULTS/ANTICIPATED RESULTS:** MENs were successfully localized to target cortical regions within 30 minutes of magnetic application. After wirelessly applying ~450 G magnetic field between 10-20 Hz, we observed a dramatic increase of calcium signals (i.e. neuronal excitability) both *in vitro* cultured neurons and *in vivo* treated animals. Whole brain imaging of awake mice showed a focal increase in calcium signals at the area where MENs localized and the signals spread to regions further away. We also found MENs stimulatory effects lasted up to 24 hours post treatment. MEN stimulation increases c-Fos expression but resulted in no inflammatory changes, up to one week, by assessing microglial or astrocytes activations. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our study shows, through controlling the applied magnetic field, MENs can be focally delivered to specific cortical regions with high efficacy and wirelessly activated neurons with high spatial and temporal resolution. This method shows promising potential to be a new non-invasive brain modulation approach disease studies and treatments.

4165

### Mechanisms of Prophage-Mediated Virulence Driving Community-Acquired MRSA Contagion

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**OBJECTIVES/GOALS:** We recently identified a CA-MRSA strain in Brooklyn, New York (USA300-BKV) causing an outbreak of severe skin infections in predominantly healthy children. The evolution of USA300-BKV included acquisition of a novel prophage, and our objective is to identify the prophage-encoded gene(s) and mechanism responsible for increased bacterial virulence. **METHODS/STUDY POPULATION:** We deleted candidate genes from a novel mosaic block of phage-encoded genes in USA300-BKV that have been shown to enhance virulence in a murine skin infection model. Deletion mutants and complemented clones will be evaluated *in vivo* to identify culprit genes and determine the effect of lineage-specific genetic variation on the phenotype. Complementary studies include a comprehensive characterization of phage and bacterial genes expressed during lysogeny *in vitro* using RNA sequencing (RNA-Seq), and *in vivo* using a targeted approach focusing on known bacterial virulence and phage lytic pathways as well as candidate genes identified by *in vitro* studies. **RESULTS/ANTICIPATED RESULTS:** Comparison of otherwise isogenic lab strains showed that the mosaic block of phage genes present in USA300-BKV enhance skin abscess size in mice, confirming previous results. As this region of the phage, named mΦ11, does not contain known toxin genes, we hypothesize that mΦ11 modulates expression of bacterial host genes to enhance virulence. Thus, transcriptional profiles of CA-MRSA containing mΦ11 and selected deletion mutants are expected to reveal changes in known or novel virulence factors compared to controls. Candidate regulators specific to the mosaic block include an adenine methyltransferase linked to changes in global gene expression of other bacterial species. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our

results will broaden scientific understanding of phage-bacterial interactions and determine the mechanisms by which phage impact virulence independent from toxin gene carriage. Identification of phage-encoded gene(s) enhancing CA-MRSA contagion will inform surveillance efforts and identify novel therapeutic targets.

4007

### Medroxyprogesterone Upregulates the Glucocorticoid Receptor in Female Long Evans Rats

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**OBJECTIVES/GOALS:** Estrogen monotherapy in postmenopausal women can reduce kidney function, while dual therapy combining estrogen with a progestin improves renal health. Using the female Long Evans rat as a novel animal model of postmenopausal cardiovascular disease, we found similar results where estrogen worsens renal health while co-administration of medroxyprogesterone acetate (MPA) was protective. MPA cross-activates glucocorticoid receptors (GR), which are targeted clinically for their anti-inflammatory actions. Therefore, our goal was to determine if estrogen monotherapy induces renal damage by increasing inflammation, while dual therapy with MPA opposes inflammation by cross-activating GR. **METHODS/STUDY POPULATION:** Female Long Evans rats underwent OVX at 11 months of age and received a subcutaneous implant containing E2, E2+MPA or vehicle for 40 days. **RESULTS/ANTICIPATED RESULTS:** Co-administration of MPA prevented the E2-induced increase in proteinuria (Veh: 0.27±0.07; E2: 3.53±1.16; E2+MPA: 1.20±0.58 mg/mg creatinine; P=0.03) and decline in glomerular filtration rate (Veh: 0.51±0.02; E2: 0.24±0.05; E2+MPA: 0.39±0.05 ml/min; P<0.01). Co-administration of MPA significantly increased renal GR transcript levels compared with E2 alone (Veh: 0.96±0.02; E2: 0.94±0.10; E2+MPA: 1.24±0.04 fold change; P<0.01). Inflammatory marker COX 2 renal transcript levels were significantly reduced by a similar degree in both mono and dual therapies compared with vehicle (Veh: 1.07±0.06; E2: 0.81±0.04; E2+MPA: 0.81±0.04 fold change; P<0.01). Neither TNF-alpha and IL-6 mRNA nor urinary beta-microglobulin levels (Veh: 1.71±0.31; E2: 2.88±0.78; E2+MPA: 3.07±1.15 mg/day; ns) were altered. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our results show that the effect of E2 on renal pro-inflammatory markers was not altered by the addition of MPA despite the significant increase in renal GR levels. Therefore, the renoprotective effects of MPA in midlife hormone therapy may be independent of renal GR-mediated changes in the immune profile.

4006

### Methionine Dependence in Cancer: From Metabolic Phenotype to Therapy

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**OBJECTIVES/GOALS:** Methionine dependence was described 45 years ago as an increased reliance on an exogenous supply of the essential amino acid methionine in most cancer cells compared to normal cells. Methionine depletion, using either synthetic diets or the enzyme methioninase, potentiates the effects of chemotherapy and radiotherapy in tumor-bearing animal models. Two main obstacles prevent methionine dependence from integrating the

clinical treatment of cancer. The first is the weight loss associated with methionine depletion therapy, increasing the risk of cachexia in patients. The second is the stubborn absence of a mechanism to explain the inability of cancer cells to adapt to low methionine levels. **METHODS/STUDY POPULATION:** To address these two obstacles, we are using an immunocompetent murine model of metastatic melanoma to compare the effects of complete methionine deprivation with a moderate, 75-80% methionine restriction similar to the one used to increase lifespan in animal models. In an effort to identify a mechanism of action, we also performed a proteomic screen of two melanoma cell lines divergent for methionine dependence under methionine stress. **RESULTS/ANTICIPATED RESULTS:** We recently showed that methionine restriction is sufficient to provide gains in treating local and metastatic lesions in vivo, without weight loss. We observed few differences in pathway activation between the two cell lines in response to methionine stress, despite proliferation being cut by half in the methionine dependent cell line. We expect that subcellular translocation events may provide further information on the molecular bases of methionine dependence. **DISCUSSION/SIGNIFICANCE OF IMPACT:** A moderate restriction in methionine is sufficient to recapitulate the benefits of methionine depletion in cancer, without weight loss. The mechanism behind this effect remains unknown. This work contributes towards the integration of methionine dependence into clinical practice and the discovery of novel drug targets.

4196

### MICROBIAL COMPOSITION DEFINES PELVIC PAIN PHENOTYPES IN REPRODUCTIVE-AGE WOMEN

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**OBJECTIVES/GOALS:** In young women, there is significant symptomatic overlap among lower urinary tract conditions, including bladder and pelvic pain, leading to misdiagnosis and delayed care. The epidemiology of pelvic pain suggests a microbial involvement, but previous studies have not definitively identified specific bacteria associated with pain diagnoses. **METHODS/STUDY POPULATION:** We examined urinary bacterial associations with specific symptom clusters, not diagnoses. Catheterized urinary samples were obtained from 78 pre-menopausal controls and cases with bladder and pelvic pain. 16S next-generation sequencing (NGS) characterized urinary microbial populations; validated questionnaires quantified symptom type and severity. *K* means unsupervised clustering analysis of NGS data assigned subjects to urotypes based on the urinary bacterial community state types. Quantitative PCR (qPCR) confirmed the NGS results and provided objective concentrations for critical taxa. Linear regression analysis confirmed the associations of bacterial concentrations and specific symptoms. **RESULTS/ANTICIPATED RESULTS:** In a pilot study of 35 reproductive-age women with a variety of complaints NGS revealed four urotypes that correlated with symptomatology. Isolated urgency incontinence was rare; the majority of subjects with symptoms complained of genitourinary pain. Bladder-specific pain (worse with filling, relieved by voiding) was associated with *Lactobacillus iners*. Asymptomatic patients almost universally had a non-iners, *Lactobacillus*-predominant microbiota. Vaginal and urethral pain unrelated to voiding correlated with increasing Enterobacteriaceae, primarily *Escherichia coli*. Detection of these species by qPCR in a validation population (n = 43) was highly