activating point mutations within the tyrosine kinase domain of ALK as the primary cause of hereditary NB, and we and others subsequently showed that these same alterations are the most common somatic single-nucleotide mutations in the sporadic forms of the disease. Crizotinib, a first-generation small molecule ATP-competitive inhibitor of the ALK tyrosine kinase, showed limited anti-tumor activity in patients with relapsed NB harboring ALK F1174 and F1245 mutations. We have demonstrated that lorlatinib, a novel ATP-competitive ALK inhibitor, overcomes this de novo resistance in preclinical models of ALK-driven NB. Recent clinical trials with lorlatinib in patients with non-small cell lung cancer harboring an ALK fusion, and in patients with NB harboring ALK mutations show the emergence of multiple or compound ALK mutations as a mechanism of resistance. We postulate that these compound mutations disrupt the interaction between and ALK and cause resistance. In this study, we employ a computational approach to model mutated ALK in complex with lorlatinib as well as ATP to understand whether the new mutations alter the affinity or mode of lorlatinib/ATP binding to ALK, and thus cause suboptimal ALK inhibition. METHODS/STUDY POPULATION: We employ methods in computational structural biology and drug design, primarily based on molecular modeling, molecular dynamics (MD), and molecular docking. Based on existing crystal structures of wildtype ALK, we model the mutations and perform MD simulations in order to characterize the activation state of the protein as well as perform ensemble docking calculations to assess the binding affinities and modes in ALK-lorlatinib and ALK-ATP complexes. RESULTS/ANTICIPATED RESULTS: We expect that the compound mutations cause resistance to lorlatinib either by lowering protein affinity for the drug or increasing the affinity for ATP. Alternatively, the compound mutations may disrupt the protein activation state, in which case ALK may no longer be active, and another protein/pathway could be driving the resistance. DISCUSSION/SIGNIFICANCE OF IMPACT: The results of this study will enable the understanding of the mechanism of resistance to lorlatinib and facilitate the design of new ALK inhibitors, or help develop more optimal and mechanism-guided therapies aimed to overcome the resistance.

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Investigation of a Series of 1,4-diaryl-pyrazolo-pyridinones as Anti-Leishmanial Agents

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OBJECTIVES/GOALS: This study was conducted in order to identify novel chemical compounds that exhibit anti-leishmanial activity and to further characterize their efficacy and toxicity in *in vitro* and *in vivo* systems in the hopes of future chemotherapeutic developments. METHODS/STUDY POPULATION: We developed a novel, target-free fluorometric high-throughput screen (HTS) to identify small molecules with anti-leishmanial activity. Screening of 10,000 small molecules from the ChemBridge DIVERset-EXP library cassette #5 yielded 210 compounds that killed 80% of parasites. One hundred nine (109) molecular scaffolds were represented within the hit compounds, including the 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP). A total of 27 novel 1,4-DAPP compounds were synthesized and anti-leishmanial efficacy and host cell toxicity was determined using L. donovani mCherry expressing amastigotes and THP-1 macrophages. Additional pharmacokinetic analyses of a potent 1,4-DAPP compound were conducted. RESULTS/ANTICIPATED RESULTS: Four experimental compounds had IC50 values less than 5μM, providing similar anti-leishmanial activity to miltefosine. Compound 9279817 had a clearance almost twice the rate of normal hepatic blood flow and had a relatively high volume of distribution, indicating this compound is rapidly cleared and distributes into tissues. *in vitro* rat liver microsome assays suggest a rapid metabolism of 9279817, and MS/MS results suggest this metabolite is most likely formed via oxidation of the sulfur on the lower aromatic ring. DISCUSSION/SIGNIFICANCE OF IMPACT: This study revealed a novel structural class of compounds that have anti-leishmanial activity. *in vitro* experiments show compounds with similar efficacy as miltefosine while having significantly less toxicity, suggesting that this class could be further developed as a potential chemotherapeutic.

4447

Leptin supplementation prevents the loss of hypoglycemia-induced glucagon release following exposure to six days of severe caloric restriction in mice

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OBJECTIVES/GOALS: We have recently shown that mice exposed to six days of 60% caloric restriction acutely display reduced hypoglycemia-induced glucagon release following refeeding, and that this effect is concurrent with low leptin levels. The current study was conducted to ascertain if leptin treatment during caloric restriction would reverse this effect. METHODS/STUDY POPULATION: Three groups of mice were used, an ad libitum (Ad-lib) fed group and two caloric restriction (CR) groups, one of which received twice daily leptin injection (0.5-1μg/g; IP) and the other vehicle (saline) during their caloric restriction. CR mice were placed on 60% caloric restriction for 6 consecutive days. Ad lib mice were housed in an identical manner but fed ad libitum during this same period. Following 6 days of restriction, CR mice were given ad lib access to food for 16 h. After the 16 h period of refeeding, both CR and ad lib mice began a 6 h fast which was immediately followed by a hypoglycemic insulin tolerance test (ITT). ITTs consisted of a variable dose of insulin intended to achieve a blood glucose of ~45 mg/dL within 60 minutes, at which time blood was collected for glucagon and corticosterone assays. RESULTS/ANTICIPATED RESULTS: The mean blood glucose levels during the ITT at 45 and 60 minutes post injection across all three groups were $46.8 + 3.1$ and $37.0 + 2.4$, respectively. There were no significant differences in glucose levels between the three groups at these two time points. As expected, saline treated CR mice displayed significantly reduced serum glucagon levels in response to the ITT relative to Ad-lib mice ($23.5 + 10.9$ vs. $91.7 + 20.8$ pg/mL, $p = 0.009$). In contrast, leptin-treated CR mice maintained their hypoglycemia-induced glucagon response to the ITT ($78.0 + 16.8$ pg/mL, $p>0.99$ vs. Ad-lib group). In addition, although corticosterone levels in saline treated CR mice were numerically lower than in Ad-lib mice, this difference was not statistically significance ($3928 + 277$ vs. $4571 + 178$ pg/mL, $p = 0.179$). DISCUSSION/SIGNIFICANCE OF IMPACT: Diabetes patients on insulin therapy often develop impaired hypoglycemic counter-regulation which can lead to life-threatening hypoglycemic complications. Our results suggest that leptin may hold promise as a

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

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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-barrium titanate (CoFe2O4–BaTiO3) for non-invasive neuromodulation†

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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

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