laterality, and treatment, as well as other data, were collected. DICOM files of CT or MRI at presentation and all subsequent follow-up imaging up to 5-years post-ICH were obtained. Using a novel semi-automated image segmentation tool developed in MATLAB by our group, ICH volumes were segmented. Using T1w MRI data, ipsi- and contralateral brain volumes were segmented using NeuroQuant, a fully automated software for MRI volumetric processing, for both initial and follow-up MRIs. Ipsilateral and global cerebral volume changes over time were calculated using initial and follow-up MRI images. Spearman rank correlation between volume changes and ICH volumes were calculated for each patient. RESULTS/ANTICIPATED RESULTS: 75 spontaneous ICH patients with adequate imaging follow-up and both early and follow-up MRI comparisons were considered. Of these, 14 patients had MRIs adequate for segmentation by NeuroQuant. There was a positive correlation ($R^2 = 0.72$, p=0.03) between ABC/2, the traditional ICH volume measuring approach, and our semi-automatic segmentation tool, with ABC/2 tending to overestimate ICH volume. There was an average of -6.51% of total brain volume loss with respect to initial brain volume at follow-up. There was a negative correlation between ICH volume and both global (Spearman rank correlation coefficient (R)=-0.714, p=0.004)) and local atrophy (R=-0.785, p=0.0009), meaning that as ICH volume increases, there is greater brain volume loss. DISCUSSION/SIGNIFICANCE OF FINDINGS: Greater ICH volume is associated with greater brain volume loss both ipsilaterally, reflected as encephalomalacia, and globally. These findings are important as encephalomalacia can result in focal neurologic deficit and other neurological symptoms over time, while global brain atrophy is associated with dementia and cognitive decline.

Clinical Trial

79696

Reversible DNA Hypermethylation of the Interleukin-15 Promoter Induces IL-15 Expression, Drives the Pathogenesis of T-Cell Large Granular Lymphocytic Leukemia (T-LGLL) and Provides a Therapeutic Approach Using 5-Azacitidine

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ABSTRACT IMPACT: This work describes, for the first time, the methylome in patients with T-LGLL, focusing on the IL-15 promoter, and clearly demonstrates that 5-azacytidine decreases IL-15 production leading to T-LGLL cell death. These results form the basis a translational clinical trial in T-LGLL that will begin accrual in 2021 OBJECTIVES/GOALS: T-LGLL is an incurable leukemia with few treatment options driven by overexpression of IL-15. Our objective is to characterize the methylation status of the IL-15 promoter in T-LGLL patients and evaluate the potential use of 5-azacytidine (5-aza) in a translational trial by studying the effect of 5-aza in vitro on IL-15 levels, and the IL-15 promoter. METHODS/STUDY POPULATION: We sorted T-LGLL patient (n=3) and normal donor (ND) samples (n=3) for CD3+/CD8+/CD5-/dim for T-LGLL immunophenotype. We analyzed DNA methylation and gene expression profiling using reduced representation bisulfite and RNA sequencing and determined differential methylation and gene expression using 1-way ANOVA analysis. To determine the functional significance of differential methylation, we evaluated MOTN-1 T-LGLL cell viability in vitro with 5-aza at increasing concentrations. Next, we evaluated IL-15 gene expression in MOTN-1 cells treated with 5-Aza versus MOTN-1 with control using western immunoblot. Finally, we exposed MOTN-1 cells to a novel IL-15 inhibitor, IBI-15, and compared cell viability against MOTN-1 cells exposed to an inactive control. RESULTS/ANTICIPATED RESULTS: There was significant differential methylation (P= 0.0178) and expression (P =0.0059) in T-LGLL patients vs ND. These data revealed significant differential hypermethylation of gene promoters, including an increase in DNA methylation of the IL-15 promoter in T-LGLL cells vs ND. In MOTN-1 cells treated in vitro with 5-Aza at 24 and 48 hours, a dose-dependent decrease in the viability of T-LGLL cells was observed, from 100% to 49.5%, p=0.037. Further, a marked decrease in IL-15 expression was observed at all concentrations of 5-aza compared to control (p=0.0001). Finally, a decrease in cell viability was observed utilizing the IL-15 inhibitor IBI-15 vs control. These results confirm that 5-aza leads to decreased transcription of the IL-15 gene, possibly due to hypomethylation of the IL-15 promoter. DISCUSSION/SIGNIFICANCE OF FINDINGS: Hypermethylation of the IL-15 promoter and subsequent increase in IL-15 is critical to the pathogenesis of T-LGLL. Inhibition of the IL-15 promoter by 5-aza leads to down-regulation of the IL-15 gene transcript, which is sufficient to induce T-LGLL cell death. Based on these results, a phase I trial will be conducted using CC-486 (oral 5-Aza) in T-LGLL.

Data Science/Biostatistics/Informatics

23154

Electrical stimulation of hippocampus and amygdala produces multiple distinct responses in human ventral temporal cortex

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ABSTRACT IMPACT: This study characterizes interactions between human limbic circuitry and ventral temporal cortex using single pulse electrical stimulation, which may inform emerging stimulation therapies for epilepsy. OBJECTIVES/GOALS: The goal of electrical brain stimulation treatment is to modulate brain network function. However, stimulation inputs to different brain sites alter the network in a variety of ways. This study examines that variability by characterizing responses in a target region while stimulating multiple other brain sites. METHODS/STUDY POPULATION: We measured voltages in intracranial EEG in 6 patients who had electrodes implanted for epilepsy monitoring. We stimulated pairs of electrodes at multiple sites in the brain with a single pulse every 5 to 7 s and measured the resulting corticocortical evoked potential (CCEP) responses in the ventral temporal cortex (VTC). Using a novel clustering method, we uncovered sets of distinct canonical

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