

CXCL10. Inhibition of their receptor CXCR3 may reduce leukocyte recruitment and ameliorate hepatitis. **METHODS/STUDY POPULATION:** To determine the functional role of the IFN- $\gamma$ -induced ligands, CXCL9 and CXCL10, in hepatic leukocyte recruitment via CXCR3 we used a prf-/- mouse infected with Lymphocytic Choriomeningitis Virus (LCMV) in our well-established model mimicking human FHL. We used AMG487, a small molecule CXCR3 antagonist, while maintaining intact IFN- $\gamma$  signaling. Mice were sacrificed 10 days after infection when mice developed features of FHL: cytopenias, organomegaly, elevated serum ferritin and sCD25, and hepatic inflammation. Hepatic inflammation was characterized using flow cytometry, liver histology and noninvasive markers of hepatitis (ALT, liver size). **RESULTS/ANTICIPATED RESULTS:** AMG487 did not ameliorate the overall disease phenotype with mice developing similar FHL characteristics compared to control, including weight loss, elevation of ALT and sIL-2r as well as degree of thrombocytopenia and anemia. There was significant reduction of recruitment of CXCR3+CD4+ T cells and B cells in mice treated with AMG487. This indicates the importance of CXCR3 receptor in humoral response in FHL hepatitis. In addition, treatment with AMG487 resulted in reduction of CXCR3 expression in hepatic inflammatory monocyte (iMonos) measured by mean fluorescence intensity (MFI). **DISCUSSION/SIGNIFICANCE:** This is the first pre-clinical experience using AMG487, a small molecule CXCR3 antagonist, to treat FHL hepatitis. AMG487 changed the hepatic inflammatory milieu, reducing CD4 T-cell and B-cell recruitment, as well as CXCR3 expression on iMonos. However, it did not ameliorate FHL hepatitis and other therapeutic approaches should be pursued.

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### Eliminating System xc- Signaling Between Astrocytes and Neurons Selectively Impairs Complex Cognition

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**OBJECTIVES/GOALS:** We aim to discover safer and more effective therapeutics for CNS disorders. Current therapeutic development is hindered by dosing out drugs for safe consumption. By identifying proteins with narrow functional roles in the brain (i.e., behavioral control), we can develop drugs targeting these proteins for improved treatment safety and efficacy. **METHODS/STUDY POPULATION:** We focused on an evolutionarily new, non-neuronal, non-synaptic glutamate signaling mechanism, system xc- (Sxc). Sxc activity was eliminated by mutating the gene Slc7a11 through pronuclear injection of zinc-finger nucleases into Sprague Dawley rat embryos to create a line of rats lacking Sxc (MSxc). To confirm Sxc mutation, we verified that tissue from MSxc rats had a complete lack of xCT, which is the regulatory subunit of Sxc that is encoded by Slc7a11. We also verified that astrocyte cultures generated from MSxc tissue lacked cystine-evoked glutamate release. Next, we measured development (body weight), CNS regulation of metabolism, and other indicators of generalized, non-specific brain function as well as behaviors that are reliant on behavioral control, such as impulse control and response inhibition. **RESULTS/ANTICIPATED RESULTS:** Eliminating Sxc was not lethal and did not impair development or produce widespread changes in brain function as is commonly observed when deleting other glutamate mechanisms. MSxc rats did not differ from wildtype in growth rate, central regulation of metabolism as reflected by absolute or diurnal changes in core body temperature, locomotor activity in a familiar or novel

environment, or simple forms of cognition such as novel object recognition, or operant responding (food and cocaine-reinforced). In contrast, behaviors that rely on behavioral control were impaired. MSxc rats displayed deficits in impulse control and behavioral flexibility. We hypothesize that MSxc rats will also show deficits in response inhibition using the stop signal reaction time task, a common metric used in clinical populations. **DISCUSSION/SIGNIFICANCE:** Eliminating Sxc activity in rats produced deficits in behaviors reliant on impulse control, without impacting development or simple brain function. These results show the potential of targeting Sxc to restore behavioral control without generating therapeutically limiting adverse effects resulting from non-specific changes in brain function.

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### Odorant exposure decreases mortality in a Dravet Syndrome mouse model

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**OBJECTIVES/GOALS:** Our goal was to explore the actions of odorants on mortality and seizures in a DS mouse model (scn1a+/-), which have spontaneous seizures and high rate of SUDEP. We hypothesize that odorants that have actions on olfactory->extended amygdala pathways will decrease SUDEP, potentially through attenuation of neuronal activation in the extended amygdala. **METHODS/STUDY POPULATION:** Dravet syndrome mice (heterozygous scn1a+/-) were exposed for at least eight hours a day to either 2-phenylethanol (2PE, rose odor), lemon extract, or vehicle odorant in group housed cages. This was repeated daily for 15 days starting at postnatal day 20/21. Mortality in each group was recorded. A subset of 2PE-exposed animals had an extended wash-out period following odorant exposure to continue to determine the long-term effect of odorant exposure on mortality. **RESULTS/ANTICIPATED RESULTS:** Our preliminary results show a strong trend for decreased mortality in the 2PE-exposed group (16.1% mortality (n=31) vs 38.5% mortality in vehicle control (n=26), p=0.06, Barnard's test). Survival analyses show similar results (p=0.056 Kaplan-Meier curve, p=0.046 when removing those animals that died before completing day one of exposure). The lemon scent-exposed animals had a non-significant increase in mortality compared to controls from our preliminary experiments (50% mortality, n=8). Overall, these results suggest that mortality effect is dependent on specific odorants and that this effect is transient. **DISCUSSION/SIGNIFICANCE:** Our preliminary data support that odorant exposure can decrease mortality in a Dravet Syndrome mouse model, suggesting that more work to determine the mechanism of action and circuitry involved may illuminate new targets and therapies for preventing SUDEP in epilepsy patients.

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### Understanding Structural and Dynamic Effects of the EWS-FLI1 interactome on the EWS Low Complexity Domain Function

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**OBJECTIVES/GOALS:** The EWSR1-FLI1 gene fusion is implicated as a source of oncogenic activity in the majority of Ewing sarcoma

(EwS) cases (>70%). Our studies will provide unparalleled insight into the transformative mechanics of EWS-FLI1. **METHODS/STUDY POPULATION:** EWS-FLI1 is an intrinsically disordered protein (IDP). IDPs do not form stable secondary or tertiary folds in biological conditions and often contain proline-rich regions and other multivalent binding regions. These regions interact with numerous partners resulting in highly dynamic and complex protein-protein interactions. Recent advances in proximity labelling techniques, such as the use of a biotin ligase fused to a protein of interest are exceptionally well suited to identifying IDP interactomes since they do not rely on the binding affinities but rather the distance between interacting proteins. Combining novel discovery proteomics with Nuclear Magnetic Resonance (NMR) approaches will provide unparalleled insight into the transformative mechanics of EWS-FLI1. **RESULTS/ANTICIPATED RESULTS:** Using the proximity labeling technique TurboID, which limits the number of false-positive interactions, we have shown that EWSR1 and

EWS-FLI1 act within the spliceosome (responsible for mRNA splicing and processing). Using bioinformatics techniques, we show EWS-FLI1 interacts with peptidyl-prolyl isomerase (PPI) proteins, specifically PPIL1, through the EWS-FLI1 N-terminal region (EWS-LCD). We used NMR to provide insight into the interaction between PPIL1 and EWS-LCD, showing that EWS-LCD interacts with the catalytic region of PPIL1. We anticipate that PPIL1 isomerizes EWS-FLI1 to modulate its activity, and that EWS-FLI1 interacts with the spliceosome through formation of a biological condensate. However, future proximity labeling and NMR studies are needed to verify this activity. **DISCUSSION/SIGNIFICANCE:** Our studies will yield actionable insights regarding the protein-protein interfaces in EWS-FLI1 that can be targeted to attenuate the oncogenic activity of EWS-FLI1. More broadly, our results will be applicable toward understanding the etiology of other pediatric cancers and for guiding the development of novel targeted treatments.