338

## Non-occupational herbicide and VOC exposures detected in dogs with multicentric lymphoma: a model for human non-Hodgkin lymphoma

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OBJECTIVES/GOALS: The objective of this study was to determine whether pet dogs with multicentric lymphoma (ML), a spontaneous, immunocompetent model for human non-Hodgkin lymphoma (NHL), are exposed to higher concentrations of herbicides and volatile organic compounds (VOCs) compared to matched unaffected control dogs. METHODS/STUDY POPULATION: We are prospectively enrolling dogs with ML within a single high-risk breed, the boxer dog, along with age-matched control boxers sampled within the same season. We are measuring urinary concentrations of the herbicides glyphosate (in Roundup®) and 2,4-D, as well as stable urinary metabolites of the VOCs benzene and 1,3-butadiene. To assess the genotoxic potential of herbicide and VOC exposures, we are using reverse dosimetry to estimate plasma exposures, and exposing healthy canine PBMC's to these concentrations of herbicides and VOCs in vitroto assess double stranded DNA damage using the Comet Chip assay. RESULTS/ANTICIPATED RESULTS: Preliminary data show significantly higher benzene exposures, measured by the stable benzene metabolite PHMA, and significantly higher 2,4-D exposures at the time of diagnosis in cases versus controls. All dogs had measurable exposures to 1,3-butadiene (measured as its stable metabolite DHBM) and glyphosate.In vitro results show significant genotoxicity thresholds of 0.1 uM for both glyphosate and 2,4-D in dog lymphoid cells. To date, these predicted plasma exposures have not been reached in vivo in boxer dogs with ML or unaffected control boxers. DISCUSSION/SIGNIFICANCE: Canine multicentric lymphoma resembles human NHL and is a potentially useful model of non-occupational chemical risk for NHL in people. The goal of this research is to identify potentially preventable non-occupational chemical risk for lymphoma and support evidence-based remediation strategies to decrease lymphoma risk in both humans and dogs.

340

## Anti-CD20 attenuates lung humoral and cellular immune responses: implications for gene therapy in Cystic Fibrosis

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OBJECTIVES/GOALS: Re-administration of inhaled gene therapies has the potential to overcome low correction efficiencies and limiting immune responses observed in previous trials of gene therapy for Cystic Fibrosis. We therefore tested the hypothesis that pre-treatment with a B-cell depleting α ± CD20 antibody would permit vector re-administration. METHODS/STUDY POPULATION: We first selected Adenoviral (Ad) vectors to study initially due to their well-known ability to elicit potent immune responses. Mice were dosed with a depleting α ± CD20 antibody or isotype control 2 days prior to delivery of Ad-Luc. 4 weeks later, mice were euthanized to assess the development of anti-vector immune responses. Flow cytometry and single-cell RNA sequencing were used to evaluate the development of lung-resident memory cells. Serum and airway antibody responses were assessed by ELISA. After 4 weeks, mice were dosed with Ad-LacZ and euthanized 3 days later to assess efficiency of second-round gene transfer by Î²-galactosidase activity assay. Similar methods were used in a pilot experiment with Adeno-associated virus vector (AAV), but with euthanasia 3 weeks after secondary gene transfer. RESULTS/ANTICIPATED RESULTS: Delivery of Ad vectors leads to the development of lung-resident memory B and T-cells. The depletion of B-cells prior to first-round vector delivery attenuated airway T-cell infiltration and serum IgG production, abrogated mucosal IgG and IgA production, and completely rescued secondary gene transfer. Genetically modified mouse models suggest secreted antibodies are critical in prevention of vector redosing. AAV vectors were found to be less immunogenic than Ad vectors, with only partial reduction of second-round gene transfer. However, anti-CD20 provided no benefit for AAV redelivery. DISCUSSION/SIGNIFICANCE: Mucosal humoral immunity is critical in preventing re-administration of Adenoviral vectors. Impairment of B-cell responses by α ± CD20 treatment prior to vector delivery allows re-administration and may help overcome low efficiencies of CF gene therapy. AAV vectors may be less susceptible to neutralization by pre-existing immunity.

342

## Impact of the Endothelial Nucleotidase on Thrombosis

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OBJECTIVES/GOALS: Contrary to current dogma, prior work has suggested that in humans, SNP-determined endothelial cell reduction in CD39 expression associates with a diminished risk for venous thromboembolism. Our objective was to examine the impact of endothelial cell (EC) CD39 expression on arterial thrombosis that replicates human data. METHODS/STUDY POPULATION: We generated a novel CD39 cell-specific conditional knockout mouse line for endothelial cells (EC-cKO: Tie2-Cre+; cd39flox/flox versus WT: Tie2-Cre-; cd39flox/flox). We validated the knockout of expression of CD39 on EC using FACS analysis and measured EC CD39 activity using the Kinase Glo ATP hydrolysis assay on magnetically sorted EC. We then used a standard FeCl3 carotid injury model to evaluate time to arterial thrombosis in vivo by measuring time to occlusion tracked via a Doppler flow probe on the exposed vessel. RESULTS/ANTICIPATED RESULTS: FACS analysis revealed a specific 97% knockout of CD39 expression on EC ($p<0.001$) but not on other cells within the vasculature. There was also significant reduction in ATP hydrolysis (81%; $p=0.019$) in EC-cKO mouse EC versus WT. We next examined the time to arterial thrombosis. EC-specific conditional knockout of CD39 exhibited a significant prolongation in time to thrombosis compared to WT (WT: 8.28 minutes +/- 0.82; EC-cKO: 11.92 minutes +/- 1.34; $p=0.024$). Analysis of carotid blood-flow revealed that EC-cKO and WT mice had similar baseline blood flow velocity ($p=0.51$), but after vessel injury with FeCl3, EC-cKO mice exhibited a 16% increase maximal flow velocity relative to baseline compared to WT ($p<0.001$), as well as a 19% increase at 2-minutes post-injury in comparison to EC-WT mice ($p<0.001$). DISCUSSION/SIGNIFICANCE: Our findings demonstrate that CD39 activity plays a role in modulating arterial thrombosis and blood-flow regulation within the vasculature. These findings

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exemplify the therapeutic potential of modulating endothelial CD39 activity, as well as the potential for using SNPs within the gene coding for CD39 as a cardiovascular disease marker.

343

## Development of osteoclast derived exosomes for vascular calcification therapy

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OBJECTIVES/GOALS: The global incidence of calcific aortic valve disease (CAVD) increased 3.5-fold since 1990. No preventative or therapeutic pharmaceutical therapies exist for CAVD. We will establish the therapeutic potential of osteoclast-derived exosomes though characterization of contents and mechanisms of action to protect against mineralization. METHODS/STUDY POPULATION: Exosomes were purified from conditioned media collected from murine myeloid precursor cells, RAW264.7 (control), and osteoclasts induced to differentiate from RAW264.7 cells (OD). Protein content of exosomes was determined using proteomic analyses. Nucleic acid contents will be identified by sequencing mRNA, miRNA, and DNA. The calcification prevention and reabsorption abilities of control and OD exosomes will be tested using human valvular interstitial cells (VIC) and smooth muscle cell calcification assays and acellular osteologic disc assays, respectively. Comparison between cellular and acellular systems will help identify mechanisms of action, and demonstrate potential therapeutic viability of OD exosomes in preventative vs resorptive treatments. RESULTS/ANTICIPATED RESULTS: OD exosomes, but not control exosomes, prevented calcification in VIC in vitro. OD exosomes contained osteoclast-specific proteins including TRAP, MMP6, cathepsin K, and bone reabsorption factors including V type proton pumps, ATPases, and integrins. These genes are also involved in resorptive activities, and were highly upregulated in OD compared to control exosomes. We anticipate miRNA signatures associated with mineral resorption will also be present. Increased knowledge of exosome cargo will illuminate their mechanism of action and allow future work to engineer increased efficacy. We also anticipate a therapeutic response when OD exosomes are applied after calcification has begun, showing exosomes promote calcium reabsorption. DISCUSSION/SIGNIFICANCE: Establishing therapeutic potential and examining mechanisms of action will pave the way for OD exosomes as a CAVD treatment. Analysis of exosome contents will determine active molecules to be enhanced in future studies. This work will lay a foundation for moving into aortic valve organoid models, which are accepted by the FDA for preclinical trials.

344

## Understanding drivers of post-Ebola syndrome (PES) in pediatric survivors of Ebolavirus disease: characterization and the way forward.

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OBJECTIVES/GOALS: Ebolavirus disease survivors report persistent, debilitating health concerns dubbed Post-Ebola Syndrome (PES). Attention to PES in young survivors is lacking, we describe PES in pediatric EVD survivors in Eastern Sierra Leone. Additionally, we introduce our proposal investigating differential presentations of PES in pediatric survivors. METHODS/STUDY POPULATION: EVD survivors were enrolled a median of 2.5 years after resolution of disease. Survivors were eligible if listed in a national register maintained by the Sierra Leone Association of Ebola Survivors. Household contacts (HCs) were identified by survivors. Participants were assigned into three comparison groups: pediatric (7-11), adolescent (12-17) and young adult (18-25). A self-reported symptom questionnaire, and a physical exam were conducted. Variables were clustered within organ system and compared across groups. RESULTS/ANTICIPATED RESULTS: Pediatric survivors had lower levels of long-term sequelae compared to adolescents and young adults. Symptoms and abnormal physical exam signs increase with age. Musculoskeletal, psychiatric, ophthalmologic, and GI signs and symptoms were significantly different between groups. Pediatric survivors had significantly more persistent sequelae than age-matched HCs with no history of EVD; particularly within the cardiac/GI (p=.006) and psychiatric/neurological (p=.025) clusters. PES is heterogeneous with respect to age, calling for a deeper understanding of age-based differences. Even the youngest group of survivors experienced significantly more sequelae than HCs, highlighting the elevated symptom burden in these children over their peers. DISCUSSION/SIGNIFICANCE: Understanding mechanistic drivers will ultimately improve targeted treatments for PES. We will characterize symptom groups defining PES in children, determine the relationship between accelerated aging and PES in this population, and test how immune profiles associated with accelerated aging relate to the development of PES in children.

345

## Fostering academic-community research teams to conduct community-engaged research in environmental justice communities: The RISE Communities R25 program

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OBJECTIVES/GOALS: Residents of environmental justice (EJ) communities experience significantly higher rates of negative health outcomes associated with poor air quality. Low-cost air sensors may supplement regulatory monitoring to better measure air pollution at local scales, but widespread application of this technology remains limited due to many challenges. METHODS/STUDY POPULATION: To address these obstacles, we designed a training program to equip community and academic research partners with the skills and knowledge to successfully apply low-cost sensors in community-engaged environmental health research. The R esearch Innovations using Sensor Technology in Environmental Justice Communities (RISE Communities) program was established through an NIEHS R25 award in 2022 and has three specific aims: 1) Foster community-academic partnerships through research education, training, and team development activities, 2) Provide technical training in the application of low-cost sensors for indoor, outdoor, and personal air monitoring in EJ communities, and 3) Establish a community of practice to address air quality in communities nationwide. RESULTS/ANTICIPATED RESULTS: We hosted our first cohort in August 2023, training five community-academic research teams in team collaboration, community-engaged research, and technical skills for collecting and analyzing data from PurpleAir sensors. Each team received 12 sensors to take to their home EJ

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