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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.

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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.

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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

https://doi.org/10.1017/cts.2024.303 Published online by Cambridge University Press