a NOAEL of 0.01 mg/kg/day were determined from IgM antibody production. These, and additional data, will support an assessment of the potential health risk to people who recreate at the Nellis Dunes site.

PS 1713s Reducing Immunogenicity in a T Cell-Dependent Antibody Response (TDAR) in Cynomolgus Monkeys Leads to a Sensitive Assessment of Immunosuppression by Abatacept (CTLA4-Ig)

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The TDAR response is used to evaluate drug effects on the immune system, assessing antigen presentation and T and B lymphocyte function. Cynomolgus monkeys injected subcutaneously on 5 occasions over 15 days with 1 mg Keyhole Limpet Hemocyanin (KLH) in Incomplete Freund's Adjuvant (IFA) developed a robust IgM and IgG antibody response to KLH. Treatment of animals twice weekly with 8 mg/mL/kg Abatacept (CTLA4-Ig) by intravenous slow bolus injection did not diminish the antibody response. In a separate study, animals were injected with 100 ug KLH in the absence of adjuvant via intradermal injections. These animals developed a detectable IgM response with inverse titers of 25,000 ten days following primary immunization. IgG responses were detectable 14 days post primary injection with inverse titers of 12,000, and 7 days post secondary challenge with inverse titers of over 50,000. Treatment of a cohort of these animals with Abatacept led to an ablation of antibody responses. Antibody responses to KLH from animals were reduced but not ablated when treated with 1 mg/kg methotrexate via subcutaneous injection. Maximal mean IgM responses to KLH 10 days following primary immunization were reduced from 120,000 to 34,000. Maximal mean IgG responses to KLH were reduced from 1 x 10^6 to 0.66 x 10^6 with maximal responses delayed from 7 to 10 days post secondary KLH challenge. Combined, these data determine that excessive antigenic stimulation can overcome the immunosuppressive effects of Abatacept and define an alternate immunization strategy that is susceptible to immunosuppression. Utilizing an appropriate immunization strategy in TDAR assays and including biological immunosuppressant controls, this model is optimized for safety assessment of immunomodulatory biological drug products.

PS 1713t Sulforaphane Inhibits Vascular Inflammation in Mice and Prevents TNF-alpha-Induced Monocyte Adhesion to Primary Endothelial Cells through Interfering with the NF-κB Pathway

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Tumor necrosis factor alpha (TNF-α) is a critical cytokine that plays important role in regulation of the toxic effects of a variety of chemicals and toxicants. Sulforaphane, a naturally-occurring isothiocyanate present in cruciferous vegetables, has received wide attention for its potential to improve vascular function in vitro. . However, its effect in vivo and the molecular mechanism of sulforaphane at physiological concentrations remain unclear. Here, we report that a sulforaphane concentration as low as 0.5 μM significantly inhibited TNF-α-induced adhesion of monocytes to human umbilical vein endothelial cells (HUVECs). Sulforaphane also significantly suppressed TNF-α-induced production of adhesion molecules. Furthermore, sulforaphane inhibited TNF-α-induced NF-κB transcriptional activity, IκBα degradation and subsequent NF-κB p65 nuclear translocation in endothelial cells, suggesting that sulforaphane can inhibit inflammation by suppressing NF-κB signaling. In an animal study, the physiologically-relevant dose of sulforaphane (300 ppm) in a mouse diet significantly abolished TNF-α-increased ex vivo monocyte adhesion and circulating adhesion molecules and chemokines in C57BL/6 mice. Histology showed that sulforaphane treatment significantly prevented the eruption of endothelial lining in the intima layer of the aorta and preserved elastin fibers' delicate organization as shown by Verhoeff-van Gieson staining. Immunohistochemistry studies showed that sulforaphane treatment also reduced VCAM-1 and monocytes-derived F4/80-positive macrophages in the aorta of TNF-α-treated mice. In conclusion, sulforaphane at physiological concentrations protects against TNF-α-induced vascular endothelial inflammation, in both in vitro and in vivo models. This anti-inflammatory effect of sulforaphane may be, at least in part, associated with interfering with the NF-κB pathway.

PS 1713u Pulmonary Toxicity and Global Gene Expression Changes in Response to Subchronic Inhalation Exposure to Crystalline Silica in Rats

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Exposure to crystalline silica results in serious health effects, most notably, silicosis and cancer. An understanding of the mechanism(s) underlying the silica-induced pulmonary toxicity is critical for the intervention and/or prevention of adverse health effects. Rats were exposed by inhalation to silica at a concentration of 15 mg/m3, 6 hours/day, 5 days/week for 3, 6 or 12 weeks. Pulmonary toxicity and global gene expression changes were determined in the lungs of the control (air) and silica exposed rats at the end of each exposure period. Pulmonary toxicity, as evidenced by an increase in lactate dehydrogenase activity and accumulation of alveolar macrophages and infiltrating neutrophils in the bronchoalveolar lavage fluid, was seen in the rats depending on the silica exposure duration. The most severe histological changes, seen in the 12-week exposure group, consisted of chronic active inflammation, type II pneumocyte hyperplasia, and fibrosis. In addition, silica was visible in the lungs of the rats belonging to the 12-week exposure group. A significant increase in the number of neutrophils seen in the blood indicated silica-induced systemic inflammation in the rats. Microarray analysis of the global gene expression profiles of the lungs detected significant differential expression (FDR $p < 0.05$ and fold change >1.5) of 38, 77 and 99 genes in the rats exposed to silica for 3-, 6- and 12-weeks, respectively, compared to the time-matched controls. Bioinformatics analysis of the differentially expressed genes identified significant enrichment of functions, networks and pathways related to inflammation, cancer, oxidative stress, fibrosis and tissue remodeling in the lungs of the silica exposed rats. Collectively, the results of our study provided insights into the molecular mechanisms underlying pulmonary toxicity following sub-chronic exposure to crystalline silica in rats.

PS 1714 Regulation of Covalent Modification of 2-Tert-Butyl-1, 4-Benzoquinone to Keap1 through Glutathione-Mediated *S*-Transarylation

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Butylated hydroxyanisole is a phenolic antioxidant and classified class-2B carcinogen. It is readily undergoes *O*-dealkylation to produce 2-*tert*-butyl-1,4-hydroquinone (TBHQ), which readily auto-oxidizes to the electrophlic metabolite 2-*tert*-butyl-1,4-benzoquinone (TBQ). TBQ causes activation of transcription factor Nrf2 copled to *S*-arylation of its negative regulator Keap1 through thiols with low p*Ka* values (Abiko Y et al., Toxicol Appl Pharmacol 255: 32-39, 2011). In a previous study, we found that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) covalently modified with 1,2-naphthoquinone (1,2-NQ) undergoes *S*-transarylation by glutathione (GSH), resulting in a recovery of GAPDH activity through removal of the 1,2-NQ from modified GAPDH and a concomitant formation of a 1,2-NQH2-SG that readily oxidizes to 1,2-NQ-SG (Miura T et al., Chem Res Toxicol 24: 1836-1844, 2011). In the present study, we explored the possibility of GSH-dependent *S*-transarylation of Keap1-TBQ adduct. Pretreatment with L-buthionine-(*S,R*)-sulfoximine, a specific inhibitor for GSH synthesis, prior to TBQ exposure of HepG2 cells suggested that the Keap1-TBQ adduct appears to undergo GSH-mediated *S*-transarylation because the depletion of GSH in the cells affected Nrf2 activation and up-regulation of its downstream protein such as heme oxygenase-1 caused by TBQ. Supporting this, a cell-free study demonstrated that TBQ covalently bound to Keap1 with low p*Ka* thiols undergoes nucleophilic attack by GSH with a p*Ka* value of 9.12, thereby eventually eliminating Keap1 and forming GSH adducts of TB(H)Q such as TBHQ-SG, TBHQ-diSG, and TBQ-diSG as determined by LC-MS/MS analysis. These results suggest that cellular GSH plays a role in the reversible covalent modification of TBQ to Keap1 (Abiko Y and Kumagai Y., Chem Res Toxicol 26: 1080-1087, 2013).

PS 1715 An Imaging-Based RNAi Screen Identifies Novel Regulators of Nrf2 Activation

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Reactive oxygen species (ROS) are major inducers of cellular stress and cell death. The anti-oxidant response pathway is therefore a key mechanism in protecting cells against ROS-mediated toxicity. The transcription factor Nrf2 is a critical regulator of the antioxidant response pathway and fundamental in cytoprotection. The activity of Nrf2 is controlled through KEAP1-mediated ubiquitination and

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