SUPPLEMENTAL-DETAILED METHODS

Animal Studies. Two separate in-life studies were conducted in cynomolgus monkeys. In one study, cynomolgus monkeys (n=9/gender/dose group) were administered amorphous bardoxolone methyl by oral gavage, using sesame oil as the vehicle, at 5, 30, and 300 mg/kg once daily for 12 months in a GLP environment. Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Clinical observations and body weights were conducted and recorded weekly. Weight data were analyzed by calculating the area under the weight versus time curve using the linear trapezoidal method with Phoenix® WinNonLin® v.6.3 (Pharsight, Cary, NC). Blood samples for clinical chemistry evaluations were collected from all animals pretest and from all animals prior to interim (6-month) and terminal (12-month) necropsies. An additional group of monkeys for each dose group were allowed to recover for 4 weeks.

A follow-up study was conducted to assess the pharmacodynamic effects of bardoxolone methyl after a shorter dosing period. For this, female cynomolgus monkeys (n=6 for vehicle and n=12 for treated) were administered bardoxolone methyl (30 mg/kg/day) by oral gavage, using sesame oil as the vehicle, once daily for 28 days. Clinical observations and body weight were handled as above. Blood samples for clinical chemistry evaluations were collected from all animals pretest and on Day 28. Urine samples (24 hours) were collected on the same days as blood. Animal welfare for both studies was compliant with the U.S. Department of Agriculture's (USDA) Animal Welfare Act (9 CFR Parts 1, 2 and 3). The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources was followed. The protocols were both reviewed and approved by an Institutional Animal Care and Use Committee (IACUC).

Clinical and Urine Chemistries. Serum BUN and serum creatinine were determined on a commercially available clinical chemistry analyzer (Alternative Biomedical Solutions, Dallas, TX). Urine chemistries in the 28-day study were determined using a Clintek 500 urine analyzer (Siemans, Tarrytown, NY). Urine creatinine (Cayman Chemical, Ann Arbor, MI, Catalog # 500701) and albumin (Active Motif, Carlsbad, CA, Catalog # 15002) were determined using commercially available kits. Monkeys with poor urine collections or with creatinine clearance values outside of two times the standard deviation were excluded from the analysis.

Histology. Kidneys from the 12-month GLP study were fixed in neutral buffered formalin and processed for H&E staining according to standard histological techniques. Slides were evaluated by a board-certified pathologist. Slides from all animals were captured digitally using an Aperio ScanScope (Aperio, Vista, CA). Representative photomicrographs are presented at 5X and 20X.

Messenger RNA Quantification. Kidneys were collected from the 28-day study, snap-frozen in liquid nitrogen, and stored at -80ºC until analysis. Kidney tissue was prepared and analyzed as previously described using the Quantigene™ Plex 2.0 assay from Affymetrix (Santa Clara, CA) (1). A modified panel (Catalog # 312050) with targets designed against the human genome was used. A description of the panel with accession numbers can be found at http://www.panomics.com. Specifically, NQO1, SRXN1, TXNRD1, GSR, GCLC, megalin, and cubilin mRNA were quantified. All data were standardized to the internal control polymerase (RNA) II (DNA directed) polypeptide A (POLR2A) and presented as fold vehicle control.

Glutathione (GSH) Quantification. Total GSH content in kidneys was determined by using a commercially available kit (Catalog #703002) from Cayman Chemical (Ann Arbor, MI). Total GSH content was normalized to protein determined using the DC Protein Assay (Catalog#500-0112) from Biorad (Hercules, CA). Data are presented as mean nmol GSH/mg protein.

Enzyme Activity Assays. Kidney tissue from the 28-day monkey study was snap-frozen in liquid nitrogen, and stored at -80ºC until analysis. Kidney tissue was homogenized at 250 mg tissue/mL ice cold PBS (pH 7.2), containing 2 mM ethylenediaminetetraacetic acid (EDTA). Homogenates were then centrifuged at 10,000 x *g* for 10 minutes at 4°C. The supernatants were collected and stored at -80°C until analysis. Protein concentrations of tissue homogenates were determined using the Bicinchoninic Acid (BCA) Protein Assay Kit from Pierce Biotechnology (Rockford, IL, Catalog #23225). NQO1 enzyme activity was determined by quantifying the rate of reduction of 2,6-dichlorophenol-indophenol (DCPIP), as previously described (2-4). Gsr enzyme activity was determined using a commercially available kit (Cayman Chemical, Ann Arbor, MI, Catalog# 703202). All enzyme activities were normalized to protein and presented as fold vehicle control.

Immunohistochemistry. Kidney tissue from the 28-day female monkey study (n=6/group) was fixed in neutral-buffered formalin and processed according to standard histological techniques. Formalin-fixed paraffin-embedded tissue antigens were retrieved using high- or low-pH heat-mediated retrieval. After enzyme and protein blocking steps, tissues were incubated with primary antibody (megalin, Abcam, ab76969; cubilin, Santa Cruz, sc-20607; or NQO1, Abcam, ab28947) at room temperature. A horseradish peroxidase (HRP)-conjugated polymer goat anti-Rabbit IgG (Dako Carpinteria, CA , K4003) was applied to all slides either as a secondary or tertiary reagent after anti-mouse or anti-goat IgG secondary linker. Slides were then developed using 3,3-diaminobenzidine+ (DAB+) solution (Dako, Catalog# K3468), counterstained with Mayer's hematoxylin, dehydrated, and permanently cover-slipped. Representative photomicrographs are presented at 5X and 20X. For densitometry, the whole slide sections of kidney were evaluated by a board-certified pathologist, and the largest possible representative

region of cortex was demarcated by manual annotation for analytical determination of stain area and intensity. Areas of artifactually altered staining, such as tissue edge fading or stain particle aggregation were omitted from the region of analysis in each kidney section. Manually annotated regions of cortex were then analyzed using a deconvolution algorithm with threshold stain levels set to encompass all levels of stain and also to include all negative and positive staining in all background tissue area such that appropriate area percent calculations could be made. The lowest level of stain intensity (1+) was adjusted to its lowest level and utilized as background area staining, and medium positive (2+) and strong positive (3+) were used to denote actual intensity levels of positive DAB+ staining. Data are presented as percent change from vehicle-treated monkeys.

Statistics. Data presented graphically are represented as mean ± standard error of the mean (S.E.M.). Data presented in tabular form are represented as mean ± standard deviation (SD). Data were analyzed by t-test or one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range *post-hoc* test with p values <0.05 considered statistically significant. The analyses were conducted using Sigmaplot 12.0 (Systat, Inc, San Jose, CA).

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