Vertebrate Animals

Description of Procedures. The laboratory has VE-Cadherin Cre-ER mice crossed to FXN flox/flox mice to yield a tamoxifen-dependent endothelial cell FXN knockout mouse model. Mice used for this study will include male and female 10- to 12-week old pathogen-free C57BL6 mice and weighing 25 grams (Charles River). Mice will be exposed to chronic hypoxia+SU-5416 or normoxia+SU-5416 for 4 weeks. After 4 weeks, mice will undergo echocardiography and right heart catheterization, followed by euthanasia and tissue harvest.

Each treatment cohort will require 20 rodents in order to account for the various types of downstream phenotypic testing. Animals will be housed at the University of Pittsburgh BST Animal Facility. All procedures and euthanasia will be performed in accordance with Institutional Animal Care and Use Committee (IACUC) approved protocols.

Justifications. C57BL6 strain of mice was chosen, as this purebred strain has been traditionally acceptable for experimental comparisons. Live hosts are necessary for these experiments because cell culture assays alone do not accurately model pulmonary hypertension, as seen in complex organisms. Two separate rodent models of PH are typically required to ensure that results of treatment are applicable broadly across multiple subtypes of this disease.

Minimization of Pain and Distress. For all animal procedures, anesthesia will be accomplished by injection of ketamine (100 mg/kg intraperitoneal) and xylazine (7.5 mg/kg intraperitoneal). If distress or pain is noted at any point in experimentation, analgesic (buprenorphine 0.05mg/kg by SC injection, every 12 hr up to 24 hrs) will be administered, and if appropriate, animals will be removed from hypoxia. Rodents will be returned to hypoxia only after symptoms of distress are resolved. In severe instances, lethargy and poor grooming, fur ruffling, poor food intake, and/or 20% weight loss may occur. If the animal achieves a 10% weight loss, nutritional supplementation will be provided to boost calorie consumption and stimulate weigh gain. This will be accomplished by the replacement of standard chow with calorie fortified (3.83 kcal/gm) food stores containing: protein, 22.4%; fat 12.0%; fiber, 6.2%; carbohydrate, 45.8% (BioServ). At 20% weight loss, euthanasia will be considered. Other signs of distress or pain include decreased activity, poor body condition, abnormal or hunched body position, and/or respiratory distress. Early euthanasia will be performed when animals exhibit Body Condition Score of <2.0 out of 5.0, with or without weight loss of >20% of baseline body weight.

Euthanasia. Methods of euthanasia are consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.