A 104-Week Historical Control Oncogenicity Study with Interim Necropsies in RccHan®:WIST Rats

Protocol Summary

I. Purpose

The objective of this study was to obtain historical control data for Wistar Han (RccHan®:WIST) Rats.

II. Test System

Rationale:	Recognized by international guidelines as a recommended test system. This study was used to increase the historical database of this animal model.
Number of Animals:	150 males weighing between 130g and 210g. 150 females weighing between 100g and 170g each as measured within three (3) days of arrival. Animals were shipped from Envigo maximum security barrier 231, (Dublin, VA) facility. Each animal was implanted with a microchip bearing a unique identification number to be used in the Provantis™ data collection system.
Age at delivery:	Approximately 5-6 weeks
Age at study start:	Approximately 7-8 weeks
Duration of Acclimatization Period:	At least two (2) weeks

III. Husbandry

Conditions:	Target range of temperature: 68-79°F, Relative humidity: 30-70%, Light cycle: 12 hours light/12 hours dark. Pair-housed, when possible (animals may be housed 2 to 3/cage during acclimation and in-life depending on study design). Animals were not re-paired when a single animal remains in a cage due to mortality.
Accommodations:	Solid bottom with nonaromatic bedding. The bedding was from an approved supplier and documented in the study.
Diet:	Animals were offered DietGel®76A, Clear H_2O®, *ad libitum* for 2-3 days following receipt. Each lot of DietGel®76A is accompanied by a Certificate of Analysis and each lot number used was identified in the study records.

	The basal diet was block Lab Diet® Certified Rodent Diet #5002, PMI Nutrition International, Inc. This diet was available *ad libitum* unless designated otherwise. Each lot number used was identified in the study records.
Water:	Tap water was supplied *ad libitum* to all animals via an automatic watering system.
Water and Diet Contaminants:	There are no known contaminants in the food or water that would interfere with this study. The drinking water used was monitored for specified contaminants at periodic intervals according to specific SOPs.
Supplemental Enrichment:	Animal enrichment was provided as described in specific SOPs.

IV. Proposed Computer Systems

Provantis™	Randomization, antemortem and postmortem data collection
Niagara Framework® Software System or Siemens Environmental Monitoring System:	Temperature and humidity monitoring and notifications in vivarium, laboratory areas, storage rooms, and archives.
Protocol Familiarization System:	Facilitates electronic protocol and protocol amendment distribution and familiarization
ExyLIMS:	Sample management and test material control activities
MPI Archiving System (MArcS): Enterprise Reporting System	Archival activities
Table Production System (TPS):	Table, graph and statistics generation
Master Control QAAD:	Quality Assurance activities
SAS®:	Statistical analysis and table and graph generation
Microsoft® Office 2003/2010 Professional:	Communications, report development, and other productivity related activities
docuBridge®:	Electronic publishing system
eDocs:	Electronic document management system

V. Antemortem Study Evaluations

Cageside Observations	Conducted at least twice daily. Animals were observed for morbidity, mortality, injury, and availability of food and water. Animals in poor health were identified for further monitoring and possible euthanasia.
Detailed Clinical Observations	Observations included but not be limited to, evaluation of the skin, fur, eyes, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling, unusual behavior, and palpation of tissue masses.

Body Weights

Taken within three (3) days of arrival, at least once prior to randomization, once weekly for the first 26 weeks, and every 4 weeks thereafter.

Food Consumption

Taken once weekly for the first 26 weeks and every 4 weeks thereafter.

Ophthalmoscopic Examinations

Animals designated for interim necropsies were examined for pretest (80/gender) and 20/gender prior to their respective scheduled interim necropsy. Both slit lamp and indirect examinations were conducted at each interval.

VI. Animal Disposition and Postmortem Study Evaluations

Study Design

	Number of Animals											
	Initial		Day 15 Interim		Day 29 Interim		Day 92 Interim		Day 183 Interim		Terminal	
Group	M	F	M	F	M	F	M	F	M	F	M	F
1	150	150	20	20	20	20	20	20	20	20	70	70

Morbidity

Moribund animals were subject to the criteria and procedures as outlined in specific SOPs.

Necropsy examinations were performed seven (7) days a week. Animals that were found dead or euthanized *in extremis* after regular working hours were held between 32-40°F overnight with necropsies performed at the start of the next day.

Method of Euthanasia

Euthanasia was by carbon dioxide inhalation followed by an approved secondary method to ensure death, e.g. exsanguination.

Disposition of Study Animals

Animals had free access to drinking water but were fasted overnight prior to necropsy. All Main Study animals were euthanized and subjected to a complete necropsy examination, performed under procedures approved by a veterinary pathologist in accordance with approved procedures.

Postmortem Evaluations

Necropsy Schedule
Interim: Day 15
Interim: Day 29
Interim: Day 92
Interim: Day 183
Terminal: Days 729-730

External Evaluations

The animals were examined carefully for external abnormalities including palpable masses.

Macroscopic Evaluations

The skin was reflected from a ventral midline incision, and any subcutaneous masses were identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs

	removed, examined, and where required, placed in the appropriate fixative.
Tissue Fixation	All tissues were placed in neutral buffered formalin, except the eye including optic nerve and testes were fixed using a modified Davidson's fixative. Eyes and testes were placed into formalin after fixation. Formalin was infused into the lung via the trachea and into the urinary bladder.
Organ Weights	Body weight and the organ weights identified in the organ list table were recorded for all animals at the scheduled necropsies, and appropriate organ weight ratios were calculated relative to body weights. Paired organs were weighed together. Organs were weighed for animals found dead or euthanized *in extremis*. A combined weight of the thyroid gland with parathyroid glands was obtained. The thyroid/parathyroid gland and pituitary gland was weighed following fixation.
Microscopic Evaluation	Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on sections of tissues and from the groups and/or intervals identified in the following table and all animals found dead or euthanized *in extremis*.
Handling of Specific Tissues	Parathyroid glands cannot always be identified macroscopically. They were examined if in the plane of section and in all cases where they are noted as grossly enlarged. A regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be identified when a mass is present. Bone marrow smears were prepared for animals found dead or euthanized *in extremis*.
Additional Testing	The pathologist may use special stains and techniques as needed to aid in the diagnosis of specific lesions. If after routine sectioning, a tissue is missed, one recut was requested either by re-sectioning the tissue in the block or ordering a wet tissue recut. If the tissue was still missing, the block was not resectioned unless the missing tissue was determined to be a potential target organ. In this case, the tissue was resectioned until located or until it was determined that it was not present in the block or in wet tissue. Tissues that were unintentionally sectioned or were present in the plane with a required tissue, though not required by protocol, were examined and documented, if abnormal.

Clinical Pathology Sample Collection and Analysis

Fasting Requirements	Free access to drinking water but were fasted overnight prior to blood collection.
Blood Sample Volume	3 to 4 mL
Site of Blood Sample Collection	Vena cava after carbon dioxide inhalation
Anticoagulant	K_3EDTA for Hematology, Citrate for Coagulation, no anticoagulant for Clinical Chemistry

Special Instruction	The order of bleeding was alternated (one animal from each group, then repeating) to reduce handling and time biases.
Urine Sample Collection	Animals were placed in stainless steel metabolism cages for at least 12 hours to collect urine.

Sample Type	Parameters Evaluated
Hematology	Leukocyte count (total and absolute differential) Erythrocyte count Hemoglobin Hematocrit Mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated) Absolute reticulocytes Platelet count Blood smear preserve and stain* *Blood smear review may be performed on select animals per SOP
Coagulation	Prothrombin time Activated partial thromboplastin time Fibrinogen
Clinical Chemistry	Alkaline phosphatase Total bilirubin (with direct bilirubin if total bilirubin exceeds 1 mg/dL) Aspartate aminotransferase Alanine aminotransferase Gamma glutamyl transferase Urea nitrogen Creatinine Total protein Albumin Globulin and A/G (albumin/globulin ratio) calculated Glucose Total cholesterol Triglycerides Electrolytes (sodium, potassium, chloride) Calcium Phosphorus
Urinalysis	Volume Color and appearance Specific gravity pH Protein Glucose Bilirubin Ketones Blood Urobilinogen Microscopy of centrifuged sediment

Organs or Tissues to be Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination All Intervals
Adrenal glands	X	X	X
Aorta		X	X
Bone marrow smear		X	X
Bone with bone marrow, femur		X	X
Bone with bone marrow, sternum		X	X

Brain, cerebrum, midbrain, cerebellum, medulla/pons, olfactory bulbs	X	X	X
Clitoral gland		X	X
Coagulating glands		X	X
Epididymides		X	X
Esophagus		X	X
Eyes (with optic nerve)		X	X
GALT (Gut-Associated Lymphoid Tissue)		X	X
Gross Lesions		X	X
Harderian glands		X	X
Heart	X	X	X
Joint, tibiofemoral		X	X
Kidneys	X	X	X
Lacrimal glands, exorbital		X	X
Large intestine, cecum		X	X
Large intestine, colon		X	X
Large intestine, rectum		X	X
Larynx		X	X
Liver	X	X	X
Lung with bronchi		X	X
Lymph node, mandibular		X	X
Lymph node, mesenteric		X	X
Mammary gland (process females only)		X	X
Nose (4 sections)		X	X
Nerve, sciatic		X	X
Ovaries	X	X	X
Oviduct		X	X
Pancreas		X	X
Pharynx		X	X
Pituitary gland	X	X	X
Preputial gland		X	X
Prostate gland	X	X	X
Salivary gland, mandibular		X	X
Salivary gland, parotid		X	X
Salivary gland, sublingual		X	X
Seminal vesicles	X	X	X
Skeletal muscle, biceps femoris		X	X
Skin		X	X
Small intestine, duodenum		X	X
Small intestine, ileum		X	X
Small intestine, jejunum		X	X
Spinal cord, cervical		X	X
Spinal cord, lumbar		X	X
Spinal cord, thoracic		X	X
Spleen	X	X	X
Stomach, glandular		X	X

Stomach, nonglandular		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid gland (with parathyroid)	X	X	X
Tissue masses with regional lymph node		X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus with cervix	X	X	X
Vagina		X	X
Zymbal's gland (auditory sebaceous gland)		X	X

VII. Statistics

Statistical analysis was not performed. However, descriptive statistics, including means and standard deviations, were calculated. For continuous endpoints, descriptive statistics consisted of means, standard deviations, and group size for group and time period. For categorical endpoints, descriptive statistics consisted either of medians or incident counts for each group and time period.

VIII. Conclusion

A total of twenty-three (23) males and nineteen (19) females were found dead or euthanized *in extremis* on study with forty-one (41) of these deaths subsequent to week 46. Four (4) males and twenty (20) females developed masses primarily in the thoracic region (1 male / 14 observations) and axillary region (10 females / 142 observations). At study termination, the mean body weights were 617.9g for males and 407.2g for females. Food consumption rates remained relatively constant for each gender over the 104-week study. Clinical pathology and anatomic pathology findings were unremarkable, with animals exhibiting normal age-related changes and study termination. In conclusion, the Wistar Han animal model is considered to be an appropriate model for both short and long term studies, demonstrating low mortality, low incidence of mass development and lower body weight gain.

IX. Regulatory Compliance and Study Guidelines

Quality Assurance Department of MPI Research
Assured compliance with internal protocols and SOPs and the GLP regulations.

Good Laboratory Practice
United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations, 21 CFR Part 58

Study Guidelines
Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Academy Press, Washington, D.C., 2011

Animal Welfare
The study was performed in an AAALAC accredited laboratory and U.S. Department of Agriculture's (USDA) Animal Welfare Act (9 CFR Parts 1, 2, and 3).