6. Ophthalmic Examination: No treatment-related changes were observed.

7. Organ Weights: Females at the 3 and 10 mg/kg dose levels exhibited higher adrenal gland weights (10 and 13% increases) compared to controls. However there were no differences in organ/body weight ratios in these groups and no associated gross or microscopic abnormalities observed with respect to the adrenal gland in these animals.

8. Gross/Microscopic Pathology: No gross or microscopic changes related to treatment with RS-25,259-197 were observed.

In conclusion, the 3 mg/kg dose was the no effect dose for this study, and provides an adequate margin of safety for both the 0.3 ug/kg initial clinical dose and for the highest planned clinical dose, 120 ug/kg.

2. Palonosetron Hydrochloride: 28-Day Subcutaneous Toxicity Study in Juvenile Rats

Report No: —— Report Number 1063/18-D6154

Conducting laboratory and location: ——

Date Started: November 12, 1999

Date of Study Report: July 2000
GLP compliance: Statements of compliance with ——— Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999 and OECD Principles on Good Laboratory Practice (revised 1997, Issued January 1998) were included; however, there were no signatures.

QA- Report: Yes (X) No ()

Methods: In a 28-day subcutaneous toxicity study, neonatal/juvenile rats received palonosetron at doses of 0, 5, 15, and 25 mg/kg/day. The vehicle or drug solution was administered by the subcutaneous route for 31 days, starting at day 4 postpartum. In the clinical setting, the intravenous route of administration will be used; however, given the small size of animals in the study, the subcutaneous route was chosen. Rats (i.e., offspring) were killed and necropsied on day after the last treatment. Parental female rats received no treatment and were sacrificed following weaning of offspring.

Dosing:
species/strain: 30 time-mated female rats [Crl:CD(SD)IGSBR strain] were obtained from ——— to provide offspring for this study. The parental female rats were 8-10 weeks old and weighed at least 160 g at mating. Pregnant female rats were delivered by day 15 of gestation. These animals were allowed to deliver naturally and the day pups were first observed was designated as day 0 postpartum. On day 4 postpartum, litters were culled to 5

pups/sex/litter through random selection. Culled pups were discarded. Pups were examined on day 4 postpartum prior to allocation to the study. Offspring within each litter were allocated to the same treatment group. Litters were randomly allocated to treatment groups. The litter performance of parental female rats and the viability of offspring up to day 4 postpartum were poorer than expected with the result that less than required number of offspring were available for allocation. Accordingly, no satellite animals were allocated to the control group. The sex of each offspring was recorded on days 1 and 4 postpartum prior to allocation to treatment groups. Sexes were confirmed at weaning (day 21 postpartum) and prior to necropsy (day 34 postpartum).

- **#/sex/group or time point:** In the main study groups, there were 10 juvenile rats/sex/group.
- **age:** Rats were 4 days of age at the start of treatment.
- **weight:** On day 4 postpartum, mean body weights ranged from 7.3 to 8.3 g for male rats and 6.3 to 7.9 g for female rats.
- **satellite groups used for toxicokinetics or recovery:** In toxicokinetic groups that received palonosetron at 5, 15, or 25 mg/kg/day, there were 15 or 16 juvenile rats/sex/group.
- **dosage groups in administered units:** 0, 5, 15, and 25 mg/kg/day
- **route, form, volume, and infusion rate:** the subcutaneous route using a dose volume of 5 ml/kg administered Vehicle or drug solution.

Drug, lot#, radiolabel, and % purity: Palonosetron, batch number 30893-P105 (Purity 99.4%)
Formulation/vehicle: The vehicle was sodium chloride and sodium phosphate adjusted to pH 7.4 with sodium hydroxide or hydrochloric acid in Water for Irrigation.
Observations and times:

- **Clinical signs:** Parental animals and offspring were monitored daily for clinical signs of toxicity. During the treatment period, pups were observed immediately and at 1 and 4 hr after dosing. Animals were observed for moribundity/mortality twice per day. Offspring that died or were sacrificed in a moribund condition during the treatment period were submitted to a macroscopic examination.
- **Body weights:** Pup body weights were measured on day 1 postpartum and daily during the treatment period (days 4 to 34 postpartum).
- **Food consumption:** Food consumption was not measured due to the age of the animals.
- **Ophthalmoscopy:** Not performed.
- **EKG:** Not performed.
- **Hematology:** Not performed.
- **Clinical chemistry:** Not performed.
- **Urinalysis:** Not performed.
- **Gross pathology:** On the day following the last dose, surviving offspring were sacrificed and submitted to necropsy examination.
- **Organs weighed:** Organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, pituitary gland, prostate gland, spleen, testes + epididymides, and thyroids + parathyroids.
- **Histopathology:** Gross lesions, principally at injection sites, from all animals and tissues from the control and high dose groups were embedded in paraffin wax BP, sectioned at 5 µm, stained with hematoxylin and eosin, and examined by a pathologist. Deaths and moribund

sacrifices during the treatment period were considered unrelated to treatment, and histopathological examination was not extended to these animals. Tissues examined from the control and high dose groups were as follows: adrenal glands, brain, cecum, colon, duodenum, eyes, femur with bone marrow and articular surfaces, heart, ileum, jejunum, kidneys, liver, lungs with mainstem bronchi, mammary (females only), mandibular lymph nodes, mesenteric lymph nodes, esophagus, optic nerves, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerves, skin, spinal cord (cervical, lumbar, and thoracic), spleen, sternum with bone marrow, stomach, testes and epididymides, thymus, thyroids and parathyroid, trachea, urinary bladder, and uterus.

- **Toxicokinetics:** Blood for measurement of plasma levels of palonosetron and its metabolite, RS-17825-007, was collected on days 1 and 28 (i.e., days 4 and 32 postpartum, respectively). Blood was collected from 4 or 5 rats/sex/group at 0.5, 1, 2, and 4 hr after dosing. Blood was obtained by decapitation on day 1 and from the orbital sinus on day 28. Samples were analyzed for palonosetron and RS-17825-007 by [redacted]. Animals in toxicokinetic groups were discarded without examination following blood collection.

- **Other:** Physical and functional development of rat pups was assessed. Eye opening was evaluated starting from day 13 postpartum. Evaluation of eye opening in the majority of pups was missed in error on day 16 postpartum. Air righting was evaluated on day 17 postpartum. Pupillary reflex and auditory startle response were evaluated on day 21 postpartum.

Results:

- **Clinical signs:** For male and female rats at 15 and 25 mg/kg/day, dose-related incidences of hair coat thinning, hair loss, and sores were observed at injection sites. For rats at 15 and 25 mg/kg/day, the percentage of animals with eyes open on day 15 postpartum was slightly reduced to 27 and 25%, respectively, as compared to 41% for the controls. By day 17 postpartum, all rats in control and treatment groups had opened their eyes. The incidences of male and female rats at 15 and 25 mg/kg/day with an absent pupillary reflex on day 21 postpartum were increased as compared to controls. For male rats at 15 and 25 mg/kg/day, the pupillary reflex was absent for 30 and 20% of animals, respectively, as compared to a poor response for 10% of controls. For female rats at 15 and 25 mg/kg/day, the pupillary reflex was absent for 10 and 30% of animals, respectively, as compared to a normal reflex for 100% of female controls. The sponsor reported background control ranges for offspring having no pupillary reflex of 0-42% for males and 0-46% for females. There were no treatment-related effects on air righting ability on postpartum day 17 or auditory response on postpartum day 21.

Pupillary reflex on postpartum day 21 for rats that received palonosetron by the subcutaneous route at doses of 0, 5, 15, and 25 mg/kg/day (mean % of pups showing a response).

Dose,	Absent		Poor		Normal	
Mg/kg/day	Male	Female	Male	Female	Male	Female
0	0	0	10	0	90	100
5	0	0	0	0	100	100
15	30	10	0	0	70	90
25	20	30	0	0	80	70

- **Mortality:** There appeared to be no treatment-related mortality. Two female control rats (#123 and #124) were found dead, 1 on day 9 and 1 on day 6. Two female control rats (#121 and #130) were sacrificed in a moribund condition, 1 on day 12 and 1 on day 27. One female control rat (#125) was missing and presumed cannibalized. One female rats at 5 mg/kg/day was sacrificed in a moribund condition on day 30, due to the poor condition of its right eye following the orbital sinus bleed. Two male rats at 15 mg/kg/day (#62 and #64) were sacrificed in a moribund condition, 1 on day 7 and 1 on day 5. One male rat at 15 mg/kg/day (#61) was found dead on day 8. One male rat and one female rat at 15 mg/kg/day (#65 and #183) were found dead on day 6. One male rat and one female rat at 25 mg/kg/day (#91 and #212) died on day 30 following anesthesia used for the orbital sinus bleed. The study protocol provided by the sponsor did not describe collection of blood samples from the orbital sinuses of rats in the main study on day 30. Further, the purpose of these blood samples was not described.

- **Body weights:** Body weight gains were suppressed >10% for male treatment groups and female rats at 25 mg/kg/day. Body weights for male controls on days 4 and 34 postpartum were 7.3 and 121.0 g, respectively. Body weight gains for male rats at 5, 15, and 25 mg/kg/day were 88.6, 87.9, and 81.1% of the control, respectively. Body weights for female controls on days 4 and 34 postpartum were 7.2 and 108.6 g, respectively. Body weight gains for female rats at 5, 15, and 25 mg/kg/day were 94.3, 101.8, and 87.0% of the control, respectively.

- **Organ Weights:** Changes in adjusted spleen and liver weights were observed for male and female rats at 15 and 25 mg/kg/day; however, there were no corresponding histopathological changes.

- **Gross pathology:** For male and female rats that received palonosetron at 15 or 25 mg/kg/day, thinning of the hair coat, hair loss, and sores were evident at injection sites (i.e., right and left shoulders and right and left hips). The incidence of pelvic dilatation in the kidneys was increased for male and female rats at 25 mg/kg/day. The incidence of protruding eyes was increased for male and female treatment groups.

Gross pathological findings for rats that received palonosetron at subcutaneous doses of 0, 5, 15, or 25 mg/kg/day from days 4 to 34 postpartum.

Organ	Male rats				Female rats			
	0	5	15	25	0	5	15	25
N =	10	10	6	9	5	9	9	9
Skin + subcutis								
-sore	0	2	1	2	0	1	2	1
-fur loss	0	0	0	1	0	0	0	0
-red	0	0	0	1	0	0	0	0
Left shoulder								
-fur loss	0	0	6	9	0	0	6	9
-sore	0	0	6	8	0	0	2	7
-red	0	0	2	7	0	0	3	3
Right shoulder								
-fur loss	0	0	6	9	0	0	7	9
-sore	0	0	3	8	0	0	5	9
-red	0	0	1	8	0	0	4	3
Right hip								

-fur loss	0	0	6	9	0	0	7	9
-sore	0	0	4	9	0	0	4	7
-red	0	0	4	6	0	0	6	3
Left hip								
-fur loss	0	0	6	9	0	0	7	9
-sore	0	0	3	7	0	0	1	9
-red	0	2	4	8	0	0	7	5
Eye								
-protruding	0	2	1	4	0	1	1	2
Kidney								
-pelvic dilatation	0	0	0	4	1	0	0	2
Stomach								
-pale	0	1	2	0	1	1	1	3

- **Histopathology:** Target organs (or tissues) of toxicity were injection sites, optic nerves, and kidneys. For male and female rats at 15 and 25 mg/kg/day, histopathological changes at injection sites included mineralization, hemorrhage, acanthosis, dermatitis, and cellulitis. Cellulitis was characterized by loss of the squamous cell layer with inflammatory cell infiltration of the underlying epidermis and dermis with scab formation and infiltration into the underlying muscle. Additionally, mineralization of the hair follicles and muscle fibers was observed. For male and female rats at 25 mg/kg/day, the incidence of neuropathy (slight to minimal, focal) in the optic nerves was increased. For male and female rats at 25 mg/kg/day, the incidence of pelvic dilatation in the kidneys was increased. The incidences of focal nephropathy and papillitis were increased for female rats at 25 mg/kg/day.

Histopathological findings for rats that received palonosetron at subcutaneous doses of 0, 5, 15, or 25 mg/kg/day from days 4 to 34 postpartum.

Organ	Male rats				Female rats			
	0	5	15	25	0	5	15	25
Skin + subcutis								
N =	10	2	1	9	5	1	2	9
-hemorrhage	0	0	0	1	0	0	1	0
-dermatitis	0	2	1	2	0	1	2	1
-cellulitis	0	0	0	1	0	0	1	0
Left shoulder								
N =	0	0	6	9	0	0	7	9
-mineralization	0	0	0	5	0	0	0	6
-hemorrhage	0	0	2	6	0	0	0	2
-acanthosis	0	0	1	1	0	0	0	1
-dermatitis	0	0	2	9	0	0	5	8
-cellulitis	0	0	5	7	0	0	4	8
Right shoulder								
N =	0	0	6	9	0	0	8	9
-mineralization	0	0	0	4	0	0	0	7
-hemorrhage	0	0	2	5	0	0	0	4
-dermatitis	0	0	4	7	0	0	6	9
-cellulitis	0	0	4	7	0	0	7	9
Right hip								

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