

Toxicokinetics: Blood samples were collected from non-fated animals on Day 1 and at the end of Week 13 immediately before the first, second and third doses on the designated days.

Semenology: For each main study male, sperm number, motility and velocity was recorded on weeks 13 and 29. Each sperm sample from the control and HD groups were evaluated microscopically for sperm morphology.

Results:

Mortality:

Dose (mg/kg/d)	n	Day of Sacrifice	Reason for Sacrifice
420	2/10	80 and 201	Killed in extremis due to eye damage sustained during bleeding.

Clinical signs:

Clinical signs	Dose (mg/kg/d)			
	0	180	340	420
Paddling	0/10	10/10	10/10	10/10
Raised tail	0/10	10/10	10/10	10/10
High gait	0/10	0/10	10/10	10/10
Mouth rubbing	0/10	10/10	10/10	10/10
Hunched	0/10	2/10	5/10	10/10
Semi closed – both eyes	0/10	1/10	5/10	6/10
Swollen abdomen	0/10	0/10	0/10	4/10
Liquid feces	0/10	3/10	5/10	10/10

LD: Paddling was observed from weeks 5 – 13; raised tail: week 5 only

MD: Paddling was observed from week 3 – 13; raised tail: weeks 3 – 7; high gait: weeks 3 – 4 and week 5;

Mouth rubbing: 2 days in week 4

HD: Paddling was observed from weeks 3 – 13; raised tail: weeks 3 – 11; high gait: 3 – 6; mouth rubbing: observed on 4 days during weeks 3 and 4. These clinical signs were observed immediately after dosing.

The hunched posture, liquid feces, semi-closed eyes and swollen abdomen were observed sporadically during weeks 1 – 4.

Body weights: (g) Week 1 – body weights were recorded before first day of treatment.

Dose (mg/kg/d)	0	180	340	420
Day 1 (pre dose)	521	512	513	489
Day 91	591	581	566	526
Gain	70	69	53	37
Decrement	-	1	17	33
% Decrement	-	1	24	47
End of recovery (Day 203)	622	616	665	617

Food consumption: (g/animal/day)

Dose (mg/kg/d)	0	180	340	420
Day 1 (Pre-dose)	24	18	16	15
Day 91	24	27	26	27
↑ in food consumption	0	9	10	12
End of recovery (Day 203)	4.0	4.5	5.7	4.3

Ophthalmoscopy: No data.

Electrocardiography: No data.

Hematology: Week 12 Data.

Dose (mg/kg/d)	0	180	340	420
MCV (fl)	53.6	53.5	52.5	52.1* (3%↓)
MCH (pg)	18.4	18.1	17.8	17.4** (5%↓)
MCHC (g/dl)	34.3	33.9	33.8	33.4* (3%↓)
HDW (g/dl)	2.89	2.43*** (16%↓)	2.40*** (17%↓)	2.36*** (18%↓)
RDW (%)	13.7	12.5* (9%↓)	12.2** (11%↓)	12.0** (12%↓)
Platelet (10 ³ /mm ³)	1226	1058* (14%↓)	1103	994*** (9%↓)
MPV (fl)	6.6	7.2	7.4** (12%↑)	7.7*** (17%↑)

PDW (%)	61.4	62.4	65.6	70.1**(14%↑)
WBC (10 ³ /mm ³)	11.4	13.4	14.7	14.7
Neutrophils (10 ³ /mm ³)	1.9	2.1	3.3**(74%↑)	3.8*** (100%↑)
Neutrophils (% WBC)	16	16	23**(44%↑)	26*** (63%↑)
Lymphocytes (% WBC)	78	78	73*(6%↓)	69**(12%↓)

* p<0.05; **p<0.01; ***p<0.001; HDW – hemoglobin distribution width; RDW – red cell distribution width; PDW – platelet distribution width; MPV – mean platelet volume

Week 29 Data (End of Recovery)

Dose (mg/kg/d)	0	180	340	420
Hb (g/dl)	15.9	15.0*	15.6	14.6**(8%↓)
RBC (10 ⁶ /mm ³)	8.99	8.71	8.93	8.31*(8%↓)
PCV (%)	48.7	46.4	48.6	44.7*(8%↓)
RET (%)	2.2	2.4	2.4	2.8*(27%↑)
Lymphocytes (% WBC)	76	70	71	67*(12%↓)

* p<0.05; **p<0.01

Clinical chemistry: Week 12 Data

Dose (mg/kg/d)	0	180	340	420
AST (IU/L)	76	107	180* (2.4X↑)	190**(2.5X↑)
ALT (IU/L)	54	67	105	111*(2X↑)
Ca (mol/l)	2.58	2.63	2.65	2.66*(1X↑)

By Week 29 (end of recovery), there was no significant differences between control and treated groups.

* p<0.05; **p<0.01

Urinalysis: No data.

Organ weights: (g) Week 13 Data.

Dose (mg/kg/d)	0	180	340	420
Spleen	0.837	0.767	0.687**(18%↓)	0.704
Spleen (% of Body wt.)	0.15	0.14	0.13	0.14
Liver	11.281	11.376	11.730	12.528*(11%↑)
Liver (% of Body wt.)	2.02	2.12	2.25	2.49
Week 29 (End of Recovery)				
Liver	12.108	12.289	12.657	13.490*(11%↑)
Liver (% of Body wt.)	2.09	2.00	2.06	2.23

* p<0.05; **p<0.01

Seminology: Mean values (Week 13 Data)

Dose (mg/kg/d)	0	180	340	420
Total sperm count (10 ⁶ /ml)	21.1	10.7	9.5	8.3*(61%↓)
% motile	76.8	48.2	37.9**(51%↓)	15.5*** (80%↓)
Average path velocity (VAP) (µm/s)	142.3	127.3	84.9	67.2*(53%↓)
Straight line velocity (VSL) (µm/s)	99.8	94.5	57.9	51.0*(49%↓)
Curvilinear velocity(VCL) (µm/s)	242.0	238.5	152.5	128.2*(47%↓)
Straightness (%)	62.6	74.9	48.8	45.1(28%↓)
Abnormal sperm (%)	0.3			49.0

Week 29 (End of recovery): At the end of the recovery period, there were no significant differences between control and treated groups i.e. effects were reversed.

* p<0.05; **p<0.01; Only control and HD groups were evaluated for abnormal sperm

Gross pathology: Week 13 data – unremarkable; Week 29 data (end of recovery): unremarkable.

Histopathology: Week 13 data. Only testicular and epididymal tissues were processed.

Dose (mg/kg/d)	0	180	340	420
Testis				
Tubular atrophy – bilateral	0/10	0/10	1/10(4)	2/10 1/10(2) 1/10(3)
Cellular debris	0/10	8/10 (1)	6/10 3/10(9) 3/10(2)	8/10 6/10(1) 2/10(2)
Spermatid retention	0/10	10/10 8/10(1) 2/10(2)	8/10(1)	7/10 6/10(1) 1/10(2)
Epididymis				
Oligospermia	1/10	0/10	1/10(4)	2/10 1/10(2) 1/10(3)
Cellular debris	0/10	10/10 7/10(1) 3/10(2)	9/10 6/10(1) 1/10(2) 1/10(3)	10/10 7/10(1) 2/10(3) 1/10(4)
Week 29 data (End of recovery)	0	180	340	420
Testis				
Tubular atrophy – unilateral	0/5	1/5(1)	0/5	1/3(1)

1-minimal, 2-slight; 3-moderate, 4-marked; 5-severe

Testis Staging data – Week 13

Dose (mg/kg/d)	0	180	340	420
Total animals examined	10	10	10	10
Animals with testis examined	10	10	9	8
Total tubules examined	1638	1677	1035	916
Mean stages VII + VIII (%)	31	29	26	25
Mean stages XII + XIII (%)	19	2	0	0
Mean abnormal atrophic tubules (%)	0	24	40	32
Week 29 data (End of recovery)	0	180	340	420
Total animals examined	5	5	5	3*
Animals with testis examined	5	5	5	3
Total tubules examined	711	724	663	424
Mean stages VII + VIII (%)	27	26	28	27
Mean stages XII + XIII (%)	16	16	17	16
Mean abnormal atrophic tubules (%)	0	0	0	0

* Excludes decedent animals

Toxicokinetics:

Only plasma concentration data to demonstrate exposure was submitted. There is no AUC data for this study.

Summary of individual study findings:

Four groups of 10 male rats each were dosed orally by gavage with OGT 918 at 180, 340 and 420 mg/kg/d (total dose) administered in split doses, TID for 13 weeks followed by 16 weeks recovery. There were no drug-related deaths. Reversible body weight gain decreases were observed at all doses. Sperm count, average path velocity, straight line velocity and curvilinear velocity decreased dose dependently achieving statistical significance at the HD (61%↓ at 14X the clinical dose – mg/m²). Percent of motile sperm decreased dose dependently achieving statistical significance ≥ MD (11X the clinical dose – mg/m²). Abnormal sperm was significantly increased by 49% in HD rats relative to 0.3% for controls. At the end of the recovery period, there were no significant difference between control and treated rats with regards to sperm parameters. Tubular atrophy (bilateral) of the testis was observed in MD (1/10) and HD (2/10) rats. Oligospermia (epididymis) was observed in MD (1/10) and HD (2/10) rats. High incidence of cellular debris was observed at doses ≥ LD (6X the clinical dose – mg/m²) in both the testis

and epididymis relative to control. A high incidence of spermatid retention was also observed in the testis at $\geq 6X$ the clinical dose - mg/m². Testicular staging data (stages XII = XIII) indicate that 19% of spermatids were retained in the seminiferous epithelium of control rats. In the treated rats, spermatid retention decreased to 2% (LD) and zero for both MD and HD groups indicative of treatment-related delayed spermiation. Abnormal atrophic tubules was increased in treated rats relative to control. At the end of the recovery period, there were no abnormal atrophic tubules in the treated rats and spermatid retention in treated rats was similar to that of control. This is indicative of reversibility of spermatid and testicular changes. NOAEL could not be established due to the testicular and epididymal changes (cellular debris) and spermatid retention observed at the LD ($\geq 6X$ the clinical dose - mg/m²).

Toxicology summary:

Mouse: Four groups of 10 male and 10 female CD-1 mice were dosed with OGT 918 by gavage TID for 14 days at 240, 1200 and 2400 mg/kg/d (total dose) and at 100, 420 and 840 mg/kg/d (total dose) for 13 weeks. In the 2-week study, due to marked weight loss in HD (39X the clinical dose - mg/m²) animals, all remaining animals in the group (9/10 HD males) and (9/10 HD females) were sacrificed on Day 9. 3 HD females (TK group) were also sacrificed on Day 9. There were no drug-related deaths in the 13-week study. In the 2-week study, erythrocyte parameters (RBC-males only), HCT, HGB and MCH-females only) decreased slightly and in a dose-dependent manner achieving statistical significance in the MD (5X and 6X the clinical dose for males and females respectively - AUC) group. Slight but significant decreases in hemoglobin (10%↓) and MCHC (10%↓) were observed in HD (7X the clinical dose - AUC) females following the 13 weeks exposure. Significant and dose-dependent decreases in WBC (41% at MD) and Lymphocytes (42%↓ at MD) were observed in treated males in the 2-week study. Eosinophils were significantly decreased by 65% in MD females. Basophils and large unstained cells were significantly decreased in MD males by 50% and 40% respectively after the 2-week exposure. The WBC, lymphocytes, basophils, eosinophils and large unstained cells were not affected in the 13-week study. Dose-dependent decrease in platelets that achieved statistical significance at doses \geq MD (2X and 3X the clinical dose for males and females respectively - AUC) was observed in all mice following 13 weeks of treatment. Similarly, platelets decreased in a dose dependent manner achieving statistical significance at doses \geq LD (1X the clinical dose for males and females - AUC) following 2-weeks of treatment.

In the 2-week study, slight but significant increase (2-fold↑) in AST was observed in MD males and females whereas ALT was slightly but significantly increased only in MD females (1.4-fold↑). In the 13-week study, AST was significantly increased by 2-fold in HD males and females (4X and 7X the clinical dose for males and females respectively - AUC). This may correlate with the hepatocyte vacuolation observed in both studies.

Absolute heart weight was significantly decreased by 19% in HD (39X the clinical dose - mg/m²) males (due to decreased body wt.) in the 2-week study. Weights of the liver and spleen (megakaryocytosis) were slightly but significantly increased in the HD group. Weight of the thymus was significantly decreased in the HD group by 50% (males) and 66% (females). This correlates with the lymphocytolysis observed. Weight of the uterus was significantly decreased (55%↓) in HD females with no correlative histopathology. In the 13-week study, weights of the heart and kidney were slightly but significantly decreased by 14%, and 17% respectively in HD males (4X the clinical dose - AUC). Heart weight was also significantly decreased by 18% in MD males (2X the clinical dose - AUC). Liver weight was significantly increased in HD (7X the clinical dose - AUC) females by 32%. Brain weight was slightly but significantly increased by 6% and 8% in MD and HD females respectively. Except for the kidney (inflammatory cell foci) there

was no correlative histopathology associated with the weight changes observed in the 13-week study.

Target organs of toxicity common to both the subacute and subchronic studies were the thymus (lymphocytolysis), liver and spleen. Toxicity of the spleen manifested as megakaryocytosis and lymphoid depletion in the subacute and subchronic studies respectively. While hepatocyte vacuolation was observed in both males and females of the subacute study, this occurred only in females of the subchronic study. In addition, toxicity of the axillary lymph node (inflammation), Kidney (inflammatory cell foci, basophilic tubules - males), spinal cord (vacuolation, mineralization) and brain (vacuolation) were observed in the subchronic study. NOAEL could not be established in the subchronic study because of brain, liver and thymus histopathology at the LD ($< 1X$ the clinical dose - AUC). The target organ affected in the subacute study but not in the subchronic study is the stomach (gastritis). NOAEL could not be established for the subacute study as well because only tissues from control and HD groups were examined for the most part. For the tissues examined from all dose groups (spleen, thymus and liver), NOAEL could not be established due to histopathology findings in the spleen and thymus at the LD ($1X$ the clinical dose for males and females - AUC). The NOAEL decreases with increased duration of dosing.

Rat: Four groups of 10 male rats each were dosed orally by gavage with OGT 918 at 180, 340 and 420 mg/kg/d (total dose) administered in split doses, TID for 13 weeks followed by 16 weeks recovery. There were no drug-related deaths. Reversible body weight gain decreases were observed at all doses. Reversible decreases in sperm parameters (sperm count, average path velocity, straight line velocity and curvilinear velocity) were observed at the HD ($14X$ the clinical dose - mg/m²). Reversible decreases in percent of motile sperm was observed at \geq MD ($11X$ the clinical dose - mg/m²). Abnormal sperm was significantly increased by 49% in HD rats relative to 0.3% for controls. Tubular atrophy (bilateral) of the testis and Oligospermia (epididymis) were observed at $\geq 11X$ the clinical dose - mg/m². High incidence of cellular debris was observed at doses \geq LD ($6X$ the clinical dose - mg/m²) in both the testis and epididymis relative to control. Reversible spermatid retention and abnormal atrophic tubules were also observed in the testis at $\geq 6X$ the clinical dose - mg/m²). NOAEL could not be established due to the testicular and epididymal changes (cellular debris) and spermatid retention observed at the LD ($\geq 6X$ the clinical dose - mg/m²).

Toxicology conclusions:

In the 13-week mouse study, signals of neurotoxicity characterized by spinal cord (vacuolation, mineralization) were observed at $4X$ the clinical dose based on AUC. Minimal to slight vacuolation of the brain was observed at doses $\geq 1X$ the clinical dose - AUC (but exceeds control incidence at HD (males) - $4X$ the clinical dose). Some control mice also had this lesion. In the rat study, drug-induced effects were observed on sperm parameters, testis and epididymis at \geq LD ($6X$ the clinical dose - mg/m²). However, these sperm effects were all reversed at the end of the recovery period.

Histopathology Inventory for NDA # 21-348 X, histopathology performed: * organ weight obtained

Study	455853	455869	1514/08
Species	Mouse	Mouse	Rat
Adrenals	y*	y*	
Aorta	y	y	
Bone Marrow smear			
Bone (femur)			
Brain	y*	y*	
Cecum	y	y	
Cervix			
Colon	y	y	
Duodenum	y	y	
Epididymis	X*	X*	X*
Esophagus	X	X	
Eye			
Fallopian tube			
Gall bladder	X*	X	
Gross lesions	y	y	
Harderian gland			
Heart	y*	y*	
Ileum	y	y	
Injection site			
Jejunum	y	y	
Kidneys	y*	y*	
Lachrymal gland			
Larynx			
Liver	y*	y*	y*
Lungs			
Lymph nodes, cervical			
Lymph nodes, mandibular			
Lymph nodes, mesenteric	X	X	
Mammary Gland			
Nasal cavity			
Optic nerves	X	X	
Ovaries	X*	X*	
Pancreas	y	y	
Parathyroid	y*	y*	
Peripheral nerve			
Pharynx			
Pituitary	y*	y*	
Prostate	X*	X*	
Rectum	y	y	
Salivary gland	X*	X*	
Sciatic nerve	y	y	
Seminal vesicles	X	X	
Skeletal muscle	y	y	
Skin	y	y	
Spinal cord	y	y	
Spleen	y*	y*	y*
Sternum	X	X	
Stomach	y	y	
Testes			y*
Thymus	y*	y*	y*
Thyroid	y*	y*	
Tongue	X	X	
Trachea	X	X	
Urinary bladder	y	y	
Uterus	X*	X*	
Vagina	X	X	
Zymbal gland			

SPONSORS DISCUSSION OF PRECLINICAL DATA IN SUPPORT OF THE REVIEW OF ADVERSE EVENTS IN THE OGS ZAVESCA CLINICAL PROGRAMME

The purpose of this document is to highlight those pre-clinical data relevant to the toxicity and pharmacology of Zavesca in the central nervous system.

1 SPONSOR'S OVERALL CONCLUSION

There is no data to support a toxic effect on the central nervous system (CNS) by miglustat, either on chronic or acute exposure. The pharmacology and pharmacokinetics of miglustat provide no mechanism for a delayed or progressive toxicity to the CNS on withdrawal of miglustat following chronic or acute exposure.

The kinetics of miglustat shows no retention of the drug in the CNS. The inhibitory effect on the glucosylceramide synthase (GCS) enzyme is shown to be rapidly reversed. The regulation of GCS is unaffected by miglustat and the pattern of glycolipid expression of the brain is also unaffected.

There are no pathological or behavioral findings in any toxicity study to support an effect of miglustat on the CNS. Miglustat treatment of animal models of neuronopathic glycosphingolipid (GSL) storage disease results in increased life span, delayed symptom onset, improved behavioral performance and increased neuronal survival.

2 INTRODUCTION

Miglustat has been tested over a wide range of pre-clinical dosages in a spectrum of normal animal species (rat, mouse, dog and monkey). Miglustat also has been tested in diverse examples of animal models of genetic diseases (Sandhoff, Tay Sachs, Niemann Pick C, and Mucopolysaccharidosis type 3A). The studies in Sandhoff and Niemann Pick C diseases demonstrate that disease dependent and progressive neuronal pathology is significantly ameliorated by miglustat treatment. At no time during this extensive pre-clinical program has there been any evidence that miglustat causes any neuropathology, either at the level of behavior/symptoms, biochemistry or histology of the central nervous system (CNS).

The long-term, high-dose studies in animals provide a clear demonstration that miglustat treatment in general and miglustat dependent glycolipid depletion in particular does not cause any neuropathies.

Any long-term effects of miglustat treatment on the CNS (following withdrawal of drug) can only be explained by:

- A. Injury to CNS giving rise to clinical signs only after withdrawal of miglustat
- B. Persistence of miglustat in nervous tissue following withdrawal of the drug
- C. Long term perturbation of glycolipid metabolism

Each of these hypotheses would have detectable consequences in the pre-clinical studies: (A) would demonstrate CNS pathology particularly after long term exposure with high doses, (B) would require that miglustat had a different kinetic profile in the CNS than in the plasma. If (C) had functional consequences, these would be expected to be detected in the Functional Observation Battery in a sensitive juvenile model and in the CNS pathology and cage-side clinical observations following long-term, high dose treatment with miglustat.

Reviewer's Comments:

- (A) *There is CNS pathology after long term exposure. In the 52 week monkey study, vascular mineralization and mineralization of the brain occurred at 750 and 2000 mg/kg/d (4X and 7X the clinical dose – AUC). Necrosis of the white matter was observed in males dosed 750*

mg/kg/d (4X THE CLINICAL DOSE – AUC). Vascular mineralization of the spinal cord was observed at 7X the clinical dose – AUC.

- (B) There is no tissue distribution study with repeat dosing to determine whether the drug accumulates in nervous tissue. With the single dose ¹⁴C-OGT 924 studies (oral), highest concentration of radioactivity occurred in the GI tract, urinary bladder and kidney. Tissues with the longest elimination half-lives included the bone marrow (20.4 hr), eye – not lens (18.0 hr) and brain (17.6 hr).
- (C) The oral (gavage) juvenile developmental toxicity study in rats with the functional observation battery showed the following CNS pathology: The 20, 60 and 180 mg/kg/d represent 1X, 2X and 6X the clinical dose of 100 mg TID based on mg/m².

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Brain				10/10		2/10		6/10
Diffuse vacuolation				1/10(1) 5/10(2) 4/10(3)		1/10(1) 1/10(2)		2/10(1) 3/10(2) 1/10(3)
Sciatic nerve				10/10				2/10
Vacuolation				3/10(1) 6/10(2) 1/10(3)		3/10(1)		1/10(2) 1/10(3)
Tibial nerve				5/10				3/10
Vacuolation				3/10(1) 2/10(2)		1/10(1)		2/10(1) 1/10(3)

1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Both genders had clinical signs of head tilting to the left. However, learning, locomotor, auditory startle, righting reflex and vision were unremarkable.

3 SPONSOR'S SUMMARY PRECLINICAL TOXICITY

The brain has been examined histologically throughout a wide range of acute and chronic toxicity studies in several laboratory animal species with no evidence of a treatment-related effect due to miglustat. Clinical observations were made in these studies with no evidence of an effect on behavior or motor activity. In addition, behavioral tests were carried out on the juvenile toxicity study (Study No. WVC0024) and the nervous system was examined histologically with no evidence of an effect of treatment. Exposure of the juvenile rat to miglustat at the most vulnerable time in the development of the central and peripheral nervous system would be expected to reveal a toxicity to these organs if the test material had such a property. The maturing myelin sheaths would be particularly vulnerable. Rapid myelin synthesis is known to occur during the weanling stage. In addition the one-year rat study (Study No. PSA-91C-3490) and the one-year monkey study on the pro-drug OGT924 (Study No. P30S4078) were similarly devoid of effects on behavior or histopathology of the nervous system. The histopathological examination of the brain in the 52 week monkey study on the pro-drug, OGT 924 (perbutyrate analogue of miglustat) included all the parts of brain routinely examined in a GLP regulatory toxicity study. This level of examination would have been detailed enough to reveal a chronic lesion in the monkey brain attributable to the administration of the test material. A similar investigation in the rat showed no evidence of treatment related lesions in the brain in the one year study at doses up to 840 mg/kg/day (approximately 14x the clinical exposure).

With respect to a potential for myelin toxicity, the key factor is the lipid composition of the myelin sheath. Myelin in the CNS is approximately 70% lipid and 30% protein, with elevated concentrations of cholesterol and phospholipid. The high lipid content favors accumulation of hydrophobic toxicants. However, miglustat is known to be highly hydrophilic (partition coefficient octanol:water at pH 7.4 = 0.13) so is unlikely to accumulate within myelin sheaths. Also, if there were an effect on myelin then the long-term, high-dose studies would be expected to reveal this,

following a repeated chronic dosing period during which the myelin would have gone through several cycles.

The absence of a neurotoxic effect as a result of administration of miglustat to a range of laboratory animal species is further reinforced if one considers the findings expected due to a true myelinotoxic compound. Two examples of this are triethyltin (TET) and hexachlorophene (Haschek et al., 1998). If oral doses of TET are administered to rodents, first muscular weakness and then paralysis occurs. Continuation of treatment or giving higher doses may lead to tremors, convulsions and death. Histologically there is widespread status spongiosus of the white matter due to extensive vacuolation most prominent in the corpus callosum, cerebellum and subcortical white matter. Vacuolation also extends to the peripheral nervous system. Ultrastructurally, these vacuoles can be attributed to a myelin split at the interperiod line. These lesions are identical to those produced by hexachlorophene. These lesions are characterized as a non-vasogenic edema. None of these appearances or behavioral and motor consequences of myelin damage have been seen as a result of the administration of miglustat to a range of laboratory animal species.

3.1 SPONSOR'S OVERVIEW OF CNS FINDINGS

The tables on the next page contain the histopathology findings in the brain from the preclinical toxicity studies. This further reinforces the lack of any treatment-related effect attributable to miglustat. However, the following discussion covers any findings in the brain in longer-term toxicity studies that may benefit from further explanation in order to demonstrate the lack of effect of miglustat on the CNS.

Vacuolation of the white matter of the brain was recorded in 2 studies (Study Nos. PSA-89S-3341; WVC/024). The results are presented in the following tables. In both of these studies the vacuolation was considered to represent a common tissue processing artifact. For WVC/024, the juvenile toxicity study, an expert view (Appendix 1) was obtained which discusses this finding in detail and confirms the artifactual nature of the findings.

4 Week Oral Rat Study with Miglustat (PSA-89S-3378)

		0		420		1680	
		M	F	M	F	M	F
Microscopic observations							
<i>All animals</i>							
Brain	No. examined	15	15	15	15	15	15
	White matter vacuolisation	1	4	3	2	1	2
	Ventricles dilatation	0	0	0	0	1	0

4 Week Oral Rat Study with Miglustat (PSA-89S-3341)

		0		180		840		4200	
		M	F	M	F	M	F	M	F
Microscopic observations									
<i>All animals</i>									
Brain	No. examined	15	15	15	15	15	15	15	15
	White matter vacuolisation	5	9	13	13	15	11	5	7
	Hydrocephalus	0	0	0	0	0	0	1	0

10 Week Oral Juvenile Rat Study with Miglustat (WVC/024)

		0		20		60		180	
		M	F	M	F	M	F	M	F
Microscopic observations									
<i>Day 70</i>									
Brain	No. examined	10	10	10	10	10	10	10	10
	Diffuse vacuolation	0	0	0	10	0	2	0	6

52 Week Oral Cynomolgus Monkey Study with OGT 924 (P30S4078)

	0		750 (OGT 924)		2000 (OGT 924)	
	M	F	M	F	M	F
Microscopic observations						
<i>Premature deaths</i>						
Brain	No. examined					
No findings	2	3	1	0	2	2
Infiltrate, macrophage, pigmented	1	3	1	-	2	2
	1	0	0	-	0	0
<i>Week 53 sacrifice</i>						
Brain	No. examined					
Infiltrate, macrophage, pigmented	5	4	5	6	6	6
Infiltrate, lymphohistiocytic, perivascular	1	3	4	3	2	1
Inflammation, subacute, meningeal	4	1	2	1	3	2
Mineralisation, vascular	1	0	0	0	0	0
Mineralisation	0	0	1	0	1	0
Necrosis, white matter	0	0	1	0	2	1
Pigment, neuronal	0	0	0	1	0	0
<i>Week 61 sacrifice</i>						
Brain	No. examined					
No findings	5	5	0	0	4	4
Infiltrate, macrophage, pigmented	1	2	-	-	2	3
Infiltrate, lymphohistiocytic, perivascular	2	3	-	-	2	1
Mineralisation, vascular	2	1	-	-	1	0
Mineralisation	1	0	-	-	0	0
Pigment, neuronal	0	1	-	-	2	0
	0	1	-	-	0	0

For Study No. PSA-89S-3341, vacuolation of the white matter of the brain was observed in rats assigned to both treatment groups as well as controls. Because of inconsistent findings regarding this central nervous system (CNS) change between this study and a related G.D. Searle study, a second section of brain for each animal was processed using differing processing regimes. This revealed that the vacuolation of the brain was not increased in any treatment group compared to controls. It was considered to be an artifactual change attributed to processing solvents rather than a treatment-induced finding. As with the findings in WVC/024, the observations were considered to be consistent with the rodent CNS vacuolar change due to over long residence in routine processing solvents e.g. ethanol and isopropyl alcohol.

In summary, myelin sheath-related vacuoles can be found in the normal brain (Greenfield's Neuropathology, 1997). The vacuoles tend to occur as single round holes in the otherwise adequately fixed white matter; their distribution may be symmetrical. Their presence is considered to be associated with prolonged immersion in 70% alcohol (Wells et al. 1989). An expert review has also been carried out on brain sections from the juvenile toxicity study (WVC0024) which supports their artifactual nature.

Reviewer's Comment: The sponsor's histopathology data is very similar to the reviewer's. The only difference is that in addition to the Week 61 data, reviewer noted spinal cord demyelination/axonal swelling in 1/5 females dosed 2000 mg/kg with minimal severity.

3.2 SPONSOR'S SUMMARY OF PHARMACOKINETICS OF BRAIN EXPOSURE

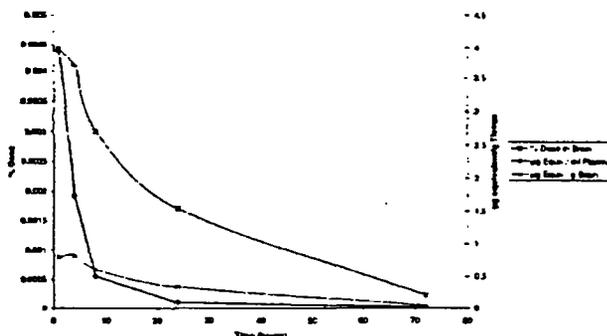
In vitro studies of cell membrane permeability have shown that miglustat is passively absorbed across CACO 2 cells (Study No. BBB-020118 1, Appendix 4) and blood-brain membrane epithelial cells (Study No. 7213-100, Appendix 5). The rate of permeability is comparable with mannitol (low permeability). Miglustat is not a substrate for p-glycoprotein or other efflux systems.

Based on these data, exposure of the CNS to miglustat would be dependent on the plasma concentration and the rate of transfer across the blood-brain barrier and that the kinetics of the drug in the CNS would follow closely the kinetics of the plasma compartment.

Tissue distribution of radiolabel following exposure of rats to ^{14}C OGT 924 137 mg/kg (Study No. MR(92B 0345). The conversion of OGT 924 to miglustat is known to be rapid and complete in the rat and the activity recorded is indicative of miglustat exposure.

One hour after administration of ^{14}C OGT 924 high levels of radioactivity were seen in the stomach and small intestine contents, medium levels were seen in the kidney and low levels were found in the liver and submaxillary glands. The other tissues or organs had the same level of radioactivity as that observed in the blood, except for the testis that were near the limit of detection and the eyes and central nervous system that were below the level of detection. Eight hours and 24 hours after administration of OGT 924 the levels of radioactivity in the CNS were near or below the limit of detection. The sponsor did not provide the LLQ for the tissue levels. Instead they stated that the LLQ is 3X the background, which is not provided.

FIGURE2: Brain Exposure to Miglustat after ^{14}C -OGT 924 Exposure
(Data from Study MRC-92S-0008)



These data suggest that there is no long-term exposure of CNS to miglustat following withdrawal of drug after single dose and that the kinetics of miglustat in the CNS is similar to its kinetics in the plasma (Report No. DWVK101 I. Appendix 6) In addition, miglustat exhibits a freely diffusable mode of access to its target enzyme and therefore recovery in GSL synthesis following drug withdrawal is rapid. This has been demonstrated in both cultured cells and in vivo (Platt et al. 1997). Study with repeat dosing has not been performed.

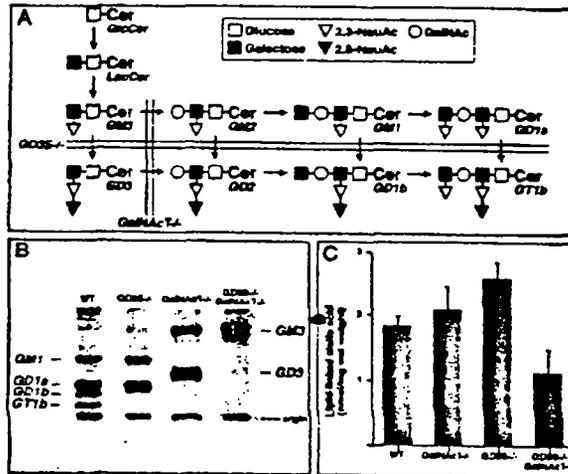
4 SPONSOR'S SUMMARY - CONSEQUENCES OF ALTERED GLYCOLIPID METABOLISM INDUCED BY MIGLUSTAT

A number of mouse strains defective in various aspects of ganglioside synthesis have been generated. These do not imitate the situation when animals are treated with miglustat and a moderate inhibition of GCS results. These models result in the complete absence of the relevant glycolipid species affected by the knockout genotype. In all cases absent gangliosides are replaced either by those normally abundant in the brain or by increased expression of a subset of typical brain gangliosides. This is summarized in Figure 3 below,. The impact upon total ganglioside content as measured by total lipid bound sialic acid determinations is remarkably small in both the GalNAcT $-/-$ and Gm synthase $-/-$ strains of mice, indeed in both cases the ganglioside content is higher than in control mice (Kawai et al. 2001).

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FIGURE 3: Illustration of the Changes in Brain Ganglioside Composition in Mice Deficient in Ganglioside Synthetic Enzymes

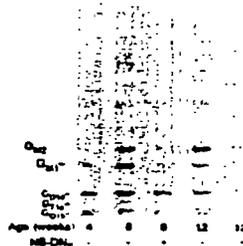
Ganglioside and MAG expression in ganglioside mutant mice. A, the partial biosynthetic pathway of gangliosides with blocks in the pathway predicted in the single and double mutant mice. B, brain ganglioside patterns from wild-type (WT) and ganglioside mutant mice. The positions of major brain gangliosides are shown on the sides of the panel. C, lipid-linked sialic acid levels from brains (n = 3) of wild-type and ganglioside mutant mice. An estimation of the total brain ganglioside concentration (nmol/10 mg wet weight) based on scanning the TLC plates and the sialic acid determination is as follows: wild-type, 7-10; *GD3S*^{-/-}, 11-16; *GalNAcT-1*^{-/-}, 12-14; *GD3S*/*GalNAcT-1*^{-/-}, 13-14.



In mice produced by crossing these two strains which therefore only express the mono sialated G_{M3} , the lipid bound sialic acid content is around half that of control mice (Kawai et al., 2001). Whilst it is true that the two strains of mice which lack the complex gangliosides typical of adult brain tissue do exhibit some neuronal defects which include progressive neuropathies in the case of the *GalNAcT-1*^{-/-} mice (Sheikh et al., 1999) and lethal audiogenic seizures in the case of the double *GalNAcT-1*^{-/-} : *GD3S* synthase^{-/-} (Kawai et al., 2001), it is also true that the brains of these mice express very high levels of either G_{M3} and G_{D3} or G_{M3} alone, those gangliosides are barely detectable in normal brain. Therefore it will be of interest to follow the studies on the *GD3S* synthase^{-/-} mice which lack b-series gangliosides but retain increased amounts of the a-series gangliosides and are therefore not devoid of complex gangliosides. Those studies published to date report no abnormalities of hind limb reflex, posture changes, spontaneous motor activity, swimming ability, flinch hearing and no sign of ataxia (Okada et al, in press). Memory and learning are also apparently normal. Histopathological examination of various brain regions also showed no abnormalities.

The neuronal defects observed in complex ganglioside knockout mice are associated with, firstly a complete absence of complex gangliosides and secondly a dramatic accumulation of simple gangliosides neither of which occur even in mice treated with high doses of miglustat (see Figure 4 below). More importantly, the abnormal behavior noted in the genetic knockout strains has not been seen in any of the preclinical studies conducted with miglustat in either GSL storage disease or normal mice.

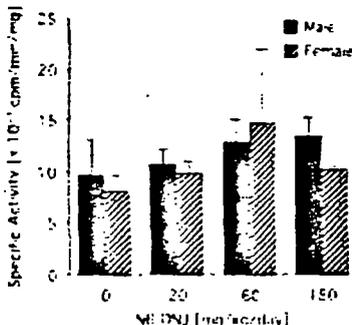
FIGURE 4: TLC Analysis of Brain Gangliosides in Tay-Sachs Mice Treated with High Dose (4800 mg/kg/day) Miglustat for 4 or 8 Weeks. (Platt et al., 1997)



The best available evidence indicates that brain levels of the drug are between 5 and 10% of those in the serum (Platt et al., 1997; Jeyakumar et al., 1999). When mice are treated with high doses of miglustat sufficient to achieve around 5 μM in the cerebrospinal fluid, the levels of brain gangliosides are reduced (see Figure 4 above) yet no drug induced gross neurological dysfunction has been observed in long term (up to 4 months) studies of both normal and GSL storage disease mice. Assuming similar drug tissue distribution in humans, the brain exposure to miglustat will be less than 1 μM . Bearing in mind that the IC_{50} for enzyme inhibition is around 20 μM , it is reasonable to assume that in a tissue with slow turnover of GSLs such as the brain, the reduction in GSL synthesis will be marginal at this dose level.

Enzyme inhibition has been demonstrated, in some cases, to lead to upregulation of enzyme. This is known to occur within 1 hour of treating MDCK cells with morpholino analogue GCS inhibitors, e.g. d,l PDMP (Abe et al., 1996) and has been reported for miglustat (Shayman et al., 2000). The mechanism for the induction in the case of morpholino analogues is thought to be due to transcriptional upregulation and is dependent upon both the inhibitor and elevation of ceramide levels. Another mechanism of enzyme upregulation could be an increase in the protein stability due to inhibitor binding. Upon drug withdrawal, upregulation of GCS could provoke a transient burst in synthesis of GlcCer over and above the normal level of synthesis which in Gaucher disease where GlcCer degradation is impaired, could result in increased GlcCer storage which might not be effectively cleared. There is data from treated rats to demonstrate that specific GCS activities in the brains of treated animals are not significantly different from controls (Figure 5).

FIGURE 5: Specific Activity of GCS in the Brains of Rats Treated for 13 Weeks with Miglustat by Oral Gavage (WV C/001).



In view of this data, we would consider it unlikely that release from enzyme inhibition particularly in tissues with very low drug exposure and slow rate of GSL turnover (e.g. brain) would result in significant over production of GlcCer. Given that type I Gaucher disease is characterized by lack of significant neuronal storage and disease even over the lifetime of a patient, it must be assumed that the turnover of GSLs is slow enough to be well within the capacity of the disabled cerebrosidease to degrade GlcCer and prevent pathological storage. Therefore, it is reasonable to assume that tissues with a slow turnover of GSLs (neurons) and low drug exposure (brain) would be less likely to suffer the consequences of a transient increase in GlcCer synthesis compared to the macrophages, which are the primary sites of GlcCer storage in type I Gaucher disease.

4.1 SPONSOR'S SUMMARY - RELATIONSHIP BETWEEN GANGLIOSIDES AND THE CNS

Where data are available, the in vivo effects of miglustat on enzyme inhibition (GSL synthesis or gastrointestinal symptoms), events of unknown mechanism (sperm abnormalities), are all completely reversible. Therefore we have no evidence that drug induced effects can be either irreversible or even persistent following drug withdrawal. In animal models of neuronopathic GSL storage diseases, although the condition is not "cured" there is a clear benefit of miglustat treatment on behavioral and phenotypic markers of disease. In such cases withdrawal of drug would presumably result in normal disease progression.

There are no studies which provide definitive proof of a critical role for ganglioside synthesis in either the initiation or progression of Alzheimer's Disease (AD), however a series of recent studies do suggest that gangliosides could have a role in the pathology of AD. The formation of insoluble β -Amyloid ($A\beta$) fibrils in the cerebral cortex is a classical pathological hallmark of Alzheimer's Disease (AD). It is thought that a conformational change in the β -Amyloid peptides to β -sheet structures is responsible for the conversion of soluble non-toxic $A\beta$ to aggregated toxic $A\beta$. However, although $A\beta$ can form fibrils, this requires concentrations of $A\beta$ that are higher than the physiological range, therefore it has been proposed that aggregation of soluble $A\beta$ requires seeded polymerization. Ganglioside GM1 has been found bound to $A\beta$ in brains exhibiting the early pathological changes of AD (Yanagisawa et al., 1995) and $A\beta$ has been shown to bind gangliosides in vitro. Recently, in vitro, studies have shown that GM1 bound $A\beta$ has a conformation different from soluble $A\beta$. Moreover, GM1 has been demonstrated to accelerate fibril formation by soluble $A\beta$ (McLaurin et al., 1998). It is now suggested that $A\beta$ recognizes and binds clusters of GM1 such as occur in detergent insoluble membrane domains (DIGs), the formation of the latter can be modulated by the cholesterol content of the membrane. Furthermore, in vitro studies using artificial lipid vesicles of varying lipid compositions indicate that increases in the cholesterol content can promote the formation of $A\beta$ /GM1 complexes (Kakio et al., 2001). In an attempt to demonstrate that the lipid composition of cellular membranes could influence AD disease pathology, the neurotoxic effects of β -amyloid peptides were assessed in neuronal cell cultures treated with agents to reduce the cholesterol and sialic acid content, both glycolipid and glycoprotein bound (Wang et al., 2001). Reduction in either membrane component was able to protect cells from the toxic effects of AD. These studies raise the possibility that GSL depletion could attenuate plaque formation and the attendant neurotoxicity.

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5 SPONSOR'S CONCLUSION

There is no data to support a toxic effect on the CNS by miglustat, either on chronic or acute exposure. The pharmacology and pharmacokinetics of miglustat provide no mechanism for a delayed or progressive toxicity to the CNS on withdrawal of miglustat following chronic or acute exposure.

The kinetics of miglustat shows no retention of the drug in the CNS. The inhibitory effect on the glucosylceramide synthase (GCS) enzyme is shown to be rapidly reversed. The regulation of GCS is unaffected by miglustat and the pattern of glycolipid expression of the brain is also unaffected.

There are no pathological or behavioral findings in any toxicity study to support an effect of miglustat on the CNS. Miglustat treatment of animal models of neuronopathic glycosphingolipid (GSL) storage disease results in increased life span, delayed symptom onset, improved behavioral performance and increased neuronal survival.

REVIEWER'S COMMENTS

There is CNS pathology after both short and long term exposure. However, the incidence of these lesions is not dose related and they do appear in some control rats as well. Dogs had clinical signs of neurotoxicity with no correlative histopathology. Rats and monkeys had no clinical signs suggestive of neurotoxicity but had histopathology findings of neurotoxicity.

SPECIES	STUDY DESIGN	TOXICITY	AUC (ng.h/ml)	mg/m ²	MULTIPLE OF THERAPEUTIC DOSE (100 mg TID)	
					AUC (ng.h/ml)	mg/m ²
Rat	OGT 918: 420, 1680 mg/kg/d, 4 weeks.	Brain: vacuolation of white matter – 5/15 (0), 5/15 (LD), 3/15 (HD)		LD: 2,520 HD: 10,080		14X 55X
	OGT 918: 180, 840, 4200 mg/kg /d, 4 weeks.	Brain: vacuolation of white matter – 14/30 (0), 26/30 (LD), 26/30 (MD), 12/30.		LD: 1,080 MD: 5,040 HD: 25,200		6X 27X 136X
Juvenile Rat	OGT 918: 20, 60, 180 mg/kg/d, PN Days 12 - 70.	Brain, sciatic/tibial nerves: vacuolation in female weanlings at ≥ 20 mg/kg/d. Both genders had head tilting to the left. Learning, locomotor, auditory startle, righting reflex and vision were unremarkable.		LD: 120 MD: 360 HD: 1080		1X 2X 6X
Dog	OGT 918: 85, 165, 495, 825 mg/kg/d, 2 weeks	Ataxia, absent/diminished pupillary, palpebral or patellar reflexes ≥ 495 mg/kg/d.		9900		≥ 54x
	OGT 918: 35, 70, 105, 140 mg/kg/d 4 weeks	Tremor, absent corneal reflexes –105 mg/kg/d.		2100		11X
Monkey	OGT 924: 750, 2000, 52 weeks	Brain: vascular mineralization, mineralization (LD & HD) & necrosis (LD) of white matter-males. Spinal cord: vascular mineralization (HD)	LD: 34,600 HD: 57,800		4X 7X	

There is no tissue distribution study with repeat dosing to determine whether the drug accumulates in nervous tissue. With the single dose ¹⁴C-OGT 924 studies (oral), highest concentration of radioactivity occurred in the GI tract, urinary bladder and kidney. Tissues with the longest elimination half-lives included the bone marrow (20.4 hr), eye – not lens (18.0 hr) and brain (17.6 hr).

The oral (gavage) juvenile developmental toxicity study in rats with the functional observation battery showed the following CNS pathology: The 20, 60 and 180 mg/kg/d represent 1X, 2X and 6X the clinical dose of 100 mg TID based on mg/m².

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Brain Diffuse vacuolation				10/10 1/10(1) 5/10(2) 4/10(3)		2/10 1/10(1) 1/10(2)		6/10 2/10(1) 3/10(2) 1/10(3)
Sciatic nerve Vacuolation				10/10 3/10(1) 6/10(2) 1/10(3)		3/10(1)		2/10 1/10(2) 1/10(3)
Tibial nerve Vacuolation				5/10 3/10(1) 2/10(2)		1/10(1)		3/10 2/10(1) 1/10(3)

1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Both genders had clinical signs of head tilting to the left. However, learning, locomotor, auditory startle, righting reflex and vision were unremarkable.

Summary of the Proposed Protocol: 13-Week Neurotoxicity Study in Rats.

Purpose: To assess the neurotoxic potential of miglustat in male and female rats when dosed TID by oral gavage for at least 90 days.

The sponsor intends to use Sprague-Dawley rats. The doses (180, 360 and 420 mg/kg/d – total dose: to be administered in split doses TID, 6 hr apart) have been selected based on previous studies conducted in the rat. The study will be conducted in accordance with OECD Principles of GLP. Animals will be assigned to 1 control and 3 treatment groups, with 15 animals/sex/group. 5 animals/sex/control and HD groups will be used for evaluation of reversibility of drug effects. All rats will be checked daily and significant changes in condition will be recorded. A functional observation battery (FOB) will be carried out. Each rat will be removed from its cage and physically examined for changes in general health status and quantitative assessments of landing foot splay, muscle weakness (fore- and hind-limb grip strength) and sensory perception (tail-flick test) will be made in weeks -1, 2, 5, 9 and 14. The observations will be made by one observer who is 'blind' with respect to the animal's treatment and recorded on a computer system by personnel not directly involved in the clinical observations.

The examination will proceed from the least to the most interactive test with observations recorded as follows:

- a) Assessment in the home cage: bizarre behavior (circling, head flicking, head searching, walking backwards, rolling over sideways, paw flicking), vocalization.
- b) Removal from the cage: approach response, response to touch (increased, decreased), vocalization.

- c) In the standard arena: activity (increased, decreased), comatosed, prostration, hunched posture, bizarre behavior (circling, head flicking, head searching, walking backwards, rolling over sideways, paw flicking), convulsions (tonic, clonic), vocalization, ataxia, tremors, reduced stability, abnormal gait, splayed gait, tiptoe gait, reduced limb function (fore, hind), upward curvature of the spine, downward curvature of the spine, piloerection, sides pinched in, ungroomed appearance, urinary incontinence, diarrhea.
- d) Handling the animal: response to touch, convulsions, vocalization, tremors, piloerection, skin color (pale, hyperemia, cyanosis), ungroomed appearance, hyperthermia, hypothermia, chromodacryorrhea, lachrymation, ptosis, endophthalmus, exophthalmus, miosis, mydriasis, stains around the mouth, stains around the nose, salivation, respiratory abnormalities (breathing rate, breathing depth, labored breathing, gasping, irregular breathing, whistling, wheezing, croaking), thin appearance, sides pinched in, dehydrated, abdominal tone (increased, decreased), urinary incontinence, diarrhea.
- e) Reflex tests: righting reflex (from supine position), response to sound (to finger click/clap), splay reflex (degree of splay when animal lifted by base of tail), visual placing response (animal is lifted by base of tail and slowly moved downwards towards the edge of arena), pupil response to light (after eye has been held closed for 10 seconds), palpebral membrane reflex (palpebral membrane touched with bristle and blink response observed), corneal reflex (hair is touched against cornea and blink reflex observed - only performed if palpebral reflex is absent), pinna reflex (bristle poked into ear canal), foot withdrawal reflex (to toe pinch).

Quantitative measures: forelimb and hind-limb grip strength, landing foot splay, time to tail flick.

Please note that the current protocol does not include a Morris Maze assessment. This has already been assessed in two rat studies as part of the developmental toxicity battery of studies with miglustat. The assessment was conducted on juvenile animals dosed from day 11 post partum to 10 weeks of age (Study WVC0024) and the F1 generation of the Pre and Post Natal Development Study (Study WVC0014). TK confirmed exposure in juvenile rat study but TK was not performed in the developmental toxicity study so it is not known if exposure occurred in pups during development.

Motor activity

Immediately after completion of the FOB, locomotor activity will be monitored by an automated activity recording apparatus that records small and large movements as an activity count. Animals will be placed individually in stainless steel cages with an infrared sensor attached and the recording session started immediately. All animals will be tested at weeks -1, 2, 5, 9 and 14.

Termination (perfusion fixed animals)

At the scheduled termination (after 13 weeks for main study and 17 weeks for the recovery groups) five designated animals/sex/group will be deeply anaesthetized with barbiturate i.p. and killed by perfusion fixation with formol saline.

Designated animals from main study groups and all recovery animals will be killed by perfusion fixation under terminal anesthesia.

Brain weight: Brain will be weighed from all animals killed by perfusion fixation.

Tissue Collection: The following tissues will be taken from all rats killed by perfusion fixation and preserved in an appropriate fixative:

Brain

Eye (with optic nerve and retina)

Spinal cord including cervical and lumbar swellings)

Spinal nerve roots (dorsal and ventral root fibers) at cervical swelling Spinal nerve roots (dorsal and ventral root fibers) at lumbar swelling Dorsal root ganglia at cervical swelling

Dorsal root ganglia at lumbar swelling

Proximal sciatic nerve

Proximal tibial nerve

Distal tibial nerve (tibial nerve calf muscle branches)

Gastrocnemius muscle

Tissue Processing: All tissues from control and high dose group animals will be processed unless indicated for storage. The following will be trimmed, embedded in paraffin wax and 5µm sections cut and stained with hematoxylin and eosin:

Transverse sections: brain (at 7 levels)

gastrocnemius muscle

eye (with retina and optic nerve)

spinal cord at cervical and lumbar swellings) to include dorsal root ganglia and spinal nerve roots (dorsal and ventral root fibers)

Longitudinal sections: spinal cord at cervical and lumbar swellings

The following will be embedded in resin and semi-thin sections cut and stained with toluidine blue:

Transverse and longitudinal sections:

proximal sciatic nerve

proximal tibial nerve

distal tibial nerve (tibial nerve calf muscle branches)

brain: cerebellum, cerebrum, mid-brain.

Tissue examination: Initially all processed tissues from the control and HD groups of the main study will be examined by light microscopy in the first instance. If treatment-related findings are observed, then affected tissues from the other treated groups may be examined and electron microscopy examination may be required.

SPONSOR'S RESPONSES TO FDA ACTION LETTER OF JUNE 20, 2002 & PHARMACOLOGY/TOXICOLOGY AND REVIEWER'S COMMENTS

FDA Comment 1

You should determine whether the adverse effects on the male reproductive function in dogs, rats and monkeys dosed with miglustat are relevant to humans through semen analysis. The specific nature, severity and reversibility of any abnormalities should be assessed.

OGS Response

In the 13-Week oral gavage toxicity study in rats, there were dose-related and statistically significant decreases in sperm count, percent motility, straightness and velocity measurements. In addition, almost half the sperm observed in the high dose (420 mg/kg/day) appeared abnormal when compared to controls. At the end of the treatment-free period reversibility of the effects was observed with the sperm appearing and behaving normally. LD = 180 mg/kg/d; MD = 340 mg/kg/d; HD = 420 mg/kg/d.

Reviewer's Comments on Sponsor's Response

Reviewer agrees with the sponsor. In the 13 week rat study with 16 week recovery period, miglustat decreased sperm count, average path velocity, straight line velocity and curvilinear velocity dose dependently achieving statistical significance at the HD. Mean total sperm count decreased by 61% at 420 mg/kg/d (14X the clinical dose – mg/m²). Percent of motile sperm decreased dose dependently achieving statistical significance ≥ MD (11X the clinical dose – mg/m²). Average path velocity (VAP), straight line velocity (VSL) and curvilinear velocity (VCL) were decreased by 53%, 49% and 47% respectively at 420 mg/kg/d (14X the clinical dose – mg/m²). Abnormal sperm was significantly increased by 49% in HD rats relative to 0.3% for controls. At the end of the recovery period, there was no significant difference between control and treated rats with regards to sperm parameters. Testicular staging data (stages XII = XIII) indicate that 19% of spermatids were retained in the seminiferous epithelium of control rats. In the treated rats, spermatid retention decreased to 2% (LD) and zero for both MD and HD groups indicative of treatment-related delayed spermiation. At the end of the recovery period, spermatid retention in treated rats was similar to that of control. This is indicative of reversibility of the drug-induced effects on sperm. The effect is drug-related at 6X the clinical dose but is reversible following 16 weeks recovery period.

Similarly in the 26 week rat study with 4-week recovery period, decreases (NS) in sperm motility, sperm concentration and normal sperm count were observed in all treated rats relative to control. These decrements were not dose related. At the end of the recovery period, while increases in these parameters were observed in most cases, they were still less than those of control suggestive of partial recovery.

WEEK 26 DATA - SUMMARY OF SPERM EVALUATION

Dose (mg/kg/d)	0	300	600	1200	Pair fed control
Sperm motility					
Total motile sperm	170 ± 69	59 ± 55(65%)	79 ± 62(54%)	50 ± 59(71%)	157 ± 63
Percent motility	41 ± 11	23 ± 16(44%)	27 ± 15(34%)	18 ± 18(56%)	42 ± 10
Sperm concentration (10⁶)					
Sperm concentration (10 ⁶)	11 ± 3	6 ± 2(36%)	8 ± 5(27%)	5 ± 3(55%)	12 ± 3
Sperm Morphology					
Normal	98 ± 2	60 ± 21(39%)	75 ± 23(24%)	55 ± 28(44%)	98 ± 2
Abnormal sperm (%)	2 ± 2	40 ± 21	26 ± 23	45 ± 28	2 ± 2
RECOVERY - SUMMARY OF SPERM EVALUATION					
Sperm motility					
Total motile sperm	162 ± 60	133 ± 38(18%)	138 ± 65(15%)	91 ± 74(44%)	201 ± 49
Percent motility	45 ± 7	45 ± 6(0%)	42 ± 11(7%)	31 ± 22(31%)	49 ± 5
Sperm concentration (10⁶)					
Sperm concentration (10 ⁶)	12 ± 4	8 ± 3(33%)	7 ± 2(42%)	5 ± 4(58%)	12 ± 2

Sperm Morphology					
Normal	98 ± 2	88 ± 9(10%)	86 ± 13(12%)	64 ± 36(35%)	98 ± 3
Abnormal sperm (%)	2 ± 2	12 ± 9	14 ± 13	36 ± 36	3 ± 3

() = % decrease relative to control

In the 52-week monkey study, sperm concentration decreased in a dose-dependent manner. Sperm evaluation was not done at the end of the recovery period. Hence the reversibility of these effects is not known.

Sperm evaluation: WEEK 52 DATA

Dose (mg/kg/d)	0 (vehicle)	750	2000
Sperm concentration ^a (10 ⁶)	7.73 ± 11.14	4.02 ± 5.41(48%)	2.96 ± 3.97 (62%)
Sperm morphology Amorphous	0.0 ± 0.0	0.0 ± 0.4	1.0 ± 1.6

^a = number of sperms/gram of caudal tissue; () = % decrease relative to control

FDA Comment 2

Neurological histopathologic assessments in animals are limited. If feasible, you should thoroughly re-evaluate brain, spine and nerve histopathology in the chronic monkey study (SA 4078). Use of special stains for neurological tissue, neuroanatomical sectioning, and ultrastructural assessments are recommended. If possible, determine ceramide and glucosylceramide plasma and/or tissue concentrations in these monkeys. A dedicated neurologic toxicity study in rodents or other suitable animal model is also recommended to evaluate ceramide and glucosylceramide levels, specific staining for various neurologic tissues (e.g., brain, spine nerve), extended neuroanatomical sectioning, and ultrastructural assessments in conjunction with a rigorous assessment of learning/memory (e.g. Morris maze).

OGS Response

The 52-week monkey study with the pro-drug (P30S4078) showed no treatment-related neuropathology. The number and location of the brain sections examined were adequate and to the industry standard for a regulatory GLP toxicity study in monkeys. Therefore, it is the view of OGS that this study provides convincing support for there being no neurotoxicity attributable to miglustat even after 52 weeks of exposure (to the pro-drug) in a non-human primate. Limited amounts of formalin fixed tissues remain from this study due to the extensive pathological examination originally conducted. In addition, the study was conducted between August 1993 and October 1994 and the condition of any remaining tissues is not likely to be suitable for further analysis after such a long period of time. There are no plasma samples remaining from this study.

Reviewer's Comments on Sponsor's Response

The sponsor stated that the 52-week monkey study with the pro-drug (SA4078) showed no treatment-related neuropathology. This is contrary to the Reviewer's observation. In the 52 week study, vascular mineralization (in brain) was observed in 1/5 LD (1X the clinical dose – AUC) and 1/6 HD (2X the clinical dose – AUC) males. Mineralization of the brain also occurred in 1/5 males at 1X the clinical dose – AUC, and in 2/6 males and 1/6 females at 2X the clinical dose – AUC). Necrosis of the white matter occurred in 1/6 males at 2X the clinical dose – AUC. Vascular mineralization (in spinal cord) was observed in 1/6 males at the clinical dose – AUC. At the end of the recovery period, 2/4 HD males and 1/5 control male had mineralization in the brain. Axonal swelling/demyelination of the spinal cord was observed in 1/4 HD female. Although assessments were of industry standard, neurologic evaluations are minimal in standard toxicity studies. Standard stains do not detect neurologic tissues and sectioning is

limited. The sponsor does not plan to evaluate the brain, spinal cord and nerve histopathology recommended for the chronic monkey study. The sponsor claim that limited amounts of formalin fixed tissues remain from this study due to the extensive pathological examination originally conducted. In addition, the study was conducted between August 1993 and October 1994 and the condition of any remaining tissues is not likely to be suitable for further analysis after such a long period of time. There are no plasma samples remaining from this study. Instead a 13-Week rat neurotoxicity study is planned.

OGS RESPONSE:

Although miglustat has not demonstrated any frank potential for neurotoxicity in preclinical studies, the concern over neurological adverse events observed in clinical trials has led the EMEA to request a further neurotoxicity study in rats. This study is to be conducted as a post approval commitment and OGS are going through the formal Protocol Assistance procedure with the EMEA to finalize the protocol. The draft protocol, which has been submitted to the EMEA, is provided in Item 5.4.2 of this submission. The protocol will be finalized by the end of March, 2003. The proposed study is a 13-week study in rats and will include investigation of neurological parameters including a Full Functional Observation Battery. The study will include perfusion fixation for detailed examination of peripheral and central nervous tissue including electron microscopy, if appropriate.

Currently, analysis of ceramide and monohexosylceramide content of tissues is not readily available as validated quantitative assays. As these lipid species exist as normal cellular components in tissues, the precise and accurate quantification of these would be essential for interpretation of the data. Therefore, any data generated on ceramide levels would be difficult to assess.

The current protocol does not include a Morris Maze assessment. This has already been assessed in two rat studies as part of the developmental toxicity battery of studies with miglustat. The assessment was conducted on juvenile animals dosed from day 11 post partum to 10 weeks of age (Study WVC0024, NDA location; Section 5.5.3.10, Vol 1.9, Page 1) and the F1 generation of the Pre and Post Natal Development Study (Study WVC0014, NDA location; Section 5.7.3.14, Vol 1.47, Page 49).

Reviewer's Comments on Sponsor's Response

Based on the concern over neurological adverse events observed in clinical trials, the Division and the EMEA recommended a further neurotoxicity study in rats to evaluate ceramide and glucosylceramide levels, specific staining for various neurologic tissues (e.g., brain, spine nerve), extended neuroanatomical sectioning, and ultrastructural assessments in conjunction with a rigorous assessment of learning/memory (e.g. Morris maze). The sponsor has agreed to conduct a 13-week study in rats as a post approval commitment to address these issues. The draft protocol for the 13-week rat study, include investigation of neurological parameters including a Full Functional Observation Battery. The study will include perfusion fixation for detailed examination of peripheral and central nervous tissue including electron microscopy, if appropriate. The sponsor stated that analysis of ceramide and monohexosylceramide content of tissues would be problematic because at present there are no validated quantitative assays for such studies. Moreover, these lipids exist as normal cellular components in tissues therefore precise and accurate quantification as well as interpretation of data generated would be difficult.

The current protocol does not include a Morris Maze assessment. The sponsor stated that this has already been assessed in two rat studies as part of the developmental toxicity battery of studies with miglustat. The assessment was conducted on juvenile animals dosed from day 11

post partum to 10 weeks of age (Study WVC0024) and the F1 generation of the Pre and Post Natal Development Study (Study WVC0014). TK was not performed to establish pup exposure to OGT 918. In study WVC0014, an E-maze learning test was conducted for the F1 generation. In study WVC0024, the Water E-maze was conducted on juvenile rats to assess the effect of the drug on learning, memory retention and swimming (locomotor function). In addition, a functional observational battery was also performed on the juvenile rats. It appears that both the Morris-maze and the Water E-maze tests evaluate learning, memory retention and swimming (locomotor function) development. Hence, the sponsor may be justified for not including a Morris Maze assessment in the protocol.

The stains mentioned in the protocol for various neurologic tissues (e.g., brain, spine nerve), include hematoxylin and eosin (brain and spinal cord – dorsal root ganglia, spinal nerve roots), and toluidine blue (proximal sciatic and tibial nerves, cerebellum, cerebrum and mid-brain). The sponsor intends to do an extended neuroanatomical sectioning (transverse and longitudinal sectioning) and ultrastructural assessments.

FDA Comment 3

Although not approvability issues per se, we have the following comments:

The results of the Caco-2 cell monolayer experiment indicated activation of P-glycoprotein by miglustat. You should provide confirmatory evidence that miglustat activates P-glycoprotein in Caco-2 cells using other substrate(s)

OGS Response

To obtain further evidence of miglustat interaction with P-glycoprotein, OGS propose to use an alternative in vitro methodology instead of carrying out further Caco-2 experiments. It is proposed to conduct a human PGP ATPase assay to test the interaction of miglustat with P-glycoprotein. The ATPase assay provides a rapid, colorimetric, compound-independent measure of the concentration dependence of any interaction of a drug with P-glycoprotein.

Reviewer's Comments on Sponsor's Response

Reviewer agrees with sponsor's proposition to use the human PGP ATPase assay to demonstrate interaction of miglustat with P-glycoprotein because this assay provides a compound-independent measure of the concentration dependence of any interaction of a drug with P-glycoprotein, but we defer to biopharm to make this assessment.

Notes From the NDA Review

Neurotoxicity:

Clinical signs and histopathology findings suggestive of neurotoxicity were observed in the dog, rat and monkey. Ataxia, diminished/absent pupillary, palpebral or patellar reflexes were observed in the dog at doses $\geq 54X$ the therapeutic dose based on mg/m^2 . In addition, tremor and absent corneal reflexes were also observed at doses $\geq 11X$ the therapeutic dose based on mg/m^2 . However, there was no histopathology finding to support the clinical signs observed in the dog. Histopathology findings in monkey brain (vascular mineralization, mineralization and necrosis of white matter) and spinal cord (vascular mineralization) were observed at doses $\geq 1X$ the therapeutic dose based on AUC with no apparent clinical signs. Vacuolation of the white matter of the brain was observed in both treated and control rats at doses $\geq 6X$ the therapeutic

dose based on mg/m². These findings were not dose-related and since control rats also had these lesions, it is not clear if it is drug related or not. Except for the lesions in the monkey brain and spinal cord, the doses at which clinical signs and histopathology findings of neurotoxicity occurred provide wide safety margins. However, neurotoxicity has been observed at the therapeutic dose in clinical trials. Weanling rats dosed postnatal days 12-70 at ≥ 20 mg/kg/d showed vacuolation of brain and sciatic/tibial nerves. Sexual development appeared slightly delayed (vaginal perforation) but locomotor, learning, righting reflex and cage behavior were unremarkable.

SIGNALS OF NEUROTOXICITY

SPECIES	STUDY DESIGN	TOXICITY	AUC (ng.h/ml)	mg/m ²	MULTIPLE OF THERAPEUTIC DOSE (100 mg TID)	
					AUC (ng.h/ml)	mg/m ²
Rat	OGT 918: 420, 1680 mg/kg/d, 4 weeks.	Brain: vacuolation of white matter – 5/15 (0), 5/15 (LD), 3/15 (HD).		LD: 2,520 HD: 10,080		14X 55X
	OGT 918: 180, 840, 4200 mg/kg /d, 4 weeks.	Brain: vacuolation of white matter – 14/30 (0), 26/30 (LD), 26/30 (MD), 12/30.		LD: 1,080 MD: 5,040 HD: 25,200		6X 27X / 136X
Juvenile Rat	OGT 918: 20, 60, 180 mg/kg/d, PN Days 12 - 70.	Brain, sciatic/tibial nerves: vacuolation in female weanlings at ≥ 20 mg/kg/d. Both genders had head tilting to the left. Learning, locomotor, auditory startle, righting reflex and vision were unremarkable.				
Dog	OGT 918: 85, 165, 495, 825 mg/kg/d, 2 weeks	Ataxia, absent/diminished pupillary, palpebral or patellar reflexes ≥ 495 mg/kg/d.		9900		≥ 54x
	OGT 918: 35, 70, 105, 140 mg/kg/d 4 weeks	Tremor, absent corneal reflexes –105 mg/kg/d.		2100		11X
Monkey	OGT 924: 750, 2000, 52 weeks	Brain: vascular mineralization, mineralization (LD & HD) & necrosis (LD) of white matter-males. Spinal cord: vascular mineralization (HD)	LD: 34,600 HD: 57,800		1X 2X	

Dogs had clinical signs of neurotoxicity without histopathology findings. Rats and monkeys had no clinical signs suggestive of neurotoxicity but had histopathology findings of neurotoxicity.

SPERM

SPECIES	STUDY DESIGN	TOXICITY	AUC (ng.h/ml)	mg/m ²	MULTIPLE OF THERAPEUTIC DOSE (100 mg TID)	
					AUC (ng.h/ml)	mg/m ²
Rat	OGT 924: 300, 600, 1200 mg/kg /d. 26 weeks	↓ Sperm motility (54%-71%), concentration (27%-54%), and # of normal sperms (23%-44%), not dose-dependent.	LD 28,400 M + F		1X	
Monkey	OGT 924: 750, 2000, 52 weeks	↓ sperm concentration 48% (LD), 62% (HD) relative to control.	LD: 34,600 HD: 57,800		1X 2X	

Sperm evaluation in Monkey: 52 week study

Dose (mg/kg/d)	0 (vehicle)	750	2000
Sperm concentration ^a (10 ⁵)	7.73 ± 11.14	4.02 ± 5.41	2.96 ± 3.97
Sperm morphology Amorphous	0.0 ± 0.0	0.0 ± 0.4	1.0 ± 1.6

^a = number of sperms/gram of caudal tissue

Sperm evaluation in the Rat: 26 week study: Week 27 data

Dose (mg/kg/d)	0	300	600	1200	Pair fed control
Sperm motility					
Total motile sperm	170 ± 69	59 ± 55	79 ± 62	50 ± 59	157 ± 63
Percent motility	41 ± 11	23 ± 16	27 ± 15	18 ± 18	42 ± 10
Sperm concentration (10 ⁵)	11 ± 3	6 ± 2	8 ± 5	5 ± 3	12 ± 3
Sperm Morphology					
Normal	98 ± 2	60 ± 21	75 ± 23	55 ± 28	98 ± 2
Amorphous	0 ± 0	3 ± 3	2 ± 4	10 ± 26	0.1 ± 0.4
Decapitated head	2 ± 2	36 ± 20	23 ± 22	33 ± 22	1 ± 2
Abnormal sperm (%)	2 ± 2	40 ± 21	26 ± 23	45 ± 28	2 ± 2
WEEK 32 (RECOVERY): SUMMARY OF SPERM EVALUATION					
Sperm motility					
Total motile sperm	162 ± 60	133 ± 38	138 ± 65	91 ± 74	201 ± 49
Percent motility	45 ± 7	45 ± 6	42 ± 11	31 ± 22	49 ± 5
Sperm concentration (10 ⁵)	12 ± 4	8 ± 3	7 ± 2	5 ± 4	12 ± 2
Sperm Morphology					
Normal	98 ± 2	88 ± 9	86 ± 13	64 ± 36	98 ± 3
Amorphous	0 ± 0	1 ± 2	0.1 ± 0.3	2 ± 3	0.1 ± 0.3
Decapitated head	2 ± 2	10 ± 10	10 ± 11	26 ± 29	2 ± 3
Abnormal sperm (%)	2 ± 2	12 ± 9	14 ± 13	36 ± 36	3 ± 3

In the male rat fertility studies, OGT 918 affected normal morphology (headless sperms, sperms with reduced hooks & miscellaneous abnormalities) and motility (VAP) of sperm (≥ 20 mg/kg/d), which was consistent with the observed reduction in fertility. Reversibility of these parameters was demonstrated on cessation of treatment (6 or 13 week recovery). In further studies on male fertility, rats were dosed and mated with untreated females. The effects on male fertility and sperm parameters were confirmed. Treatment resulted in an increase in the number of unfertilised and fragmented ova, but those that were fertilised proceeded to develop normally. When males were mated after a 6-week treatment-free period, pregnancy parameters were within normal ranges. Sperm morphology and motility was similar to that of control animals after

a 13-week treatment-free period. NOAEL could not be established because of the decreased sperm concentration, decreased number of normal sperms, increased headless sperms and increased sperms with reduced hooks in LD males. However, the LD tested is 0.6x the human dose based on mg/m².

VI. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

General Toxicology Issues:

In the subchronic mouse study, signals of neurotoxicity characterized by spinal cord (vacuolation, mineralization) were observed at 4X the clinical dose based on AUC. Vacuolation of the brain was observed at doses \geq 1X the clinical dose - AUC. Some control mice also had this lesion. In the rat study, drug-induced effects were observed on sperm parameters, testis and epididymis at \geq LD (6X the clinical dose - mg/m²). However, these effects were all reversed at the end of the recovery period.

The sponsor had proposed to conduct a 13-week rat study to evaluate the neurotoxic potential of miglustat. The current protocol does not include a Morris Maze assessment. The sponsor stated that this has already been assessed in two rat studies; one as part of the developmental toxicity battery and second with a juvenile rat study with miglustat. Reviewer agrees with sponsor. The stains mentioned in the protocol for various neurologic tissues include hematoxylin and eosin (brain and spinal cord - dorsal root ganglia, spinal nerve roots), and toluidine blue (proximal sciatic and tibial nerves, cerebellum, cerebrum and mid-brain). The sponsor intends to do an extended neuroanatomical sectioning (transverse and longitudinal sectioning) if lesions are found. An evaluation of ceramide levels is not planned.

The sponsor stated that the 52-week monkey study with the pro-drug (SA4078) showed no treatment-related neuropathology. This is contrary to the Reviewer's observation. In the 52 week study, vascular mineralization (in brain) was observed in 1/5 LD (1X the clinical dose - AUC) and 1/6 HD (2X the clinical dose - AUC) males. Mineralization of the brain also occurred in 1/5 males at 1X the clinical dose - AUC, and in 2/6 males and 1/6 females at 2X the clinical dose - AUC. Necrosis of the white matter occurred in 1/6 males at 2X the clinical dose - AUC. Vascular mineralization (in spinal cord) was observed in 1/6 males at the clinical dose - AUC. At the end of the recovery period, 2/4 HD males and 1/5 control male had mineralization in the brain. Axonal swelling/demyelination of the spinal cord was observed in 1/4 HD females. The sponsor does not plan to evaluate the brain, spinal cord and nerve histopathology recommended for the chronic monkey study. The sponsor claim that limited amounts of formalin fixed tissues remain from this study due to the extensive pathological examination originally conducted. In addition, the study was conducted between August 1993 and October 1994 and the condition of any remaining tissues is not likely to be suitable for further analysis after such a long period of time. There are no plasma samples remaining from this study.

The 13 week rat sperm analysis revealed adverse effects at \geq 340 mg/kg/d (11X the MRHD of 100 mg TID). These effects were reversible following 16 weeks recovery. Longer-term studies in rat and monkey (\geq 6 months) show similar effects at 1-2X the MRHD, but reversibility was not assessed. The sponsor plans to address this male fertility concern in product labeling because of the difficulty (religious/ethical) in obtaining reliable human sperm data in this population (Gaucher disease).

Recommendations:

None.

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/s/

John Colerangle .
6/16/03 09:51:13 AM
PHARMACOLOGIST
RESPONSE TO NOT-APPROVABLE LETTER

Karen Davis-Bruno
6/16/03 09:56:28 AM
PHARMACOLOGIST
Concurrence on P/T Supplement review + labeling

Memorandum

Date: 18 June 2002

From: David E. Morse, Ph.D.
Asc. Director (Pharm./Tox.), Office of Drug Evaluation II

To: Sandra Kweder, M.D.
Director (Acting), Office of Drug Evaluation II

Cc: David G. Orloff, M.D., Dir., DMEP (HFD-510)
Karen Davis-Bruno, Ph.D., Sup. Pharm./Tox., DMEDP (HFD-510)

Subject: NDA 21-348
ZAVESCA® — miglustat; OGT918)
Review of Pharm./Tox. Information and Sections of Proposed Product Label

I. Materials Included in Review

1. Pharm./Tox. Review of NDA 21-348, dated 8 April 2002, J. Colerangle, Ph.D.
2. Pharm./Tox. TL Memoranda, dated 10 May and 7 June 2002, K. Davis-Bruno, Ph.D.
3. CAC/ExecCAC Meeting Minutes regarding IND 60197, 18 Dec. 2001.
4. NDA 21-348 Action Package with Draft Product Labeling (date of draft product labeling not specified).

II. Background

The sponsor (Oxford Glycosciences) is seeking approval of ZAVESCA® — (miglustat; OGT918), for the chronic treatment of Type 1 Gaucher disease. Gaucher disease is caused by a failure to degrade glucosylceramide, resulting in the lysosomal storage of this material within tissue macrophages, causing widespread pathology. ZAVESCA® (miglustat) is an inhibitor of the enzyme glucosylceramide synthase, a glucosyl transferase enzyme responsible for the first step in the synthesis of most glycolipids (including glucosphingolipids). Glucosphingolipids play an important role in cell signaling, development, differentiation and host-pathogen interactions. By reducing glucosphingolipid synthesis, miglustat inhibits the excess accumulation of these lipids within cells and associated pathology. Glucosylceramide synthase is ubiquitous in tissues, which may explain the multi-tissue nature of toxicity observed and relates to the pharmacological activity of miglustat (OGT 918).

III. Comments and Conclusions

1. A review of the action package for NDA 21-348, ZAVESCA® —, indicates that the product has been extensively evaluated in multiple acute, sub-chronic and chronic repeat-dose non-clinical safety studies (including 12 month repeat-dose toxicology studies in rats and monkeys, one month repeat dose studies in mice and dogs, and standard batteries of multiple genotoxicity and reproductive toxicology studies) to support potential approval for chronic use in the treatment of patients with Type 1 Gaucher Disease (a phospholipid

storage disease). However, it should be noted that carcinogenicity studies (typically required for pharmaceuticals that are likely to be used on a chronic basis) have not been completed (rat 2-year study) or initiated (mouse study) in accordance with an agreement made between the Div. and Sponsor in Aug. 1999.

2. Review and revision of the proposed product labeling has been deferred based on the expected NA action for this application. However, it is recommended that in any future product labeling, consideration be given to the inclusion of a section on Animal Toxicology which should present the unique neurological findings observed in the repeat dose animal studies.
3. Specific comments related to the proposed pre- and post-approval toxicologic evaluation of miglustat follow:

A) Carcinogenicity testing

According to the primary P/T review, in Aug. of 1999, an agreement was reached between the Div. and Sponsor to allow for the post-approval testing of miglustat for carcinogenic potential. To date, the sponsor has submitted only one dose range finding study for concurrence by the ExecCAC on dose selection for the definitive 2-year bioassay in rats. No protocol or dose selection data have been submitted to the Div./ExecCAC for a second test species (as would generally be required for a chronic use product). It is therefore recommended that the Div. communicate with the sponsor the need for greater diligence on their part (the sponsor) to fulfill the commitment for carcinogenicity testing in a timely manner.

B) Neurotoxicology assessment in animals

The draft NA letter requests that the sponsor conduct multiple follow-up studies in animals to address the neurologic effects of chronic miglustat administration. The request for follow-up studies appear based on the clinical signs observed in patients, toxicologic effects noted in prior animal studies, and the proposed mechanism of drug action. The sponsor is specifically directed to conduct additional histopathologic assessments of specimens derived from the chronic dosing study conducted in monkeys (study SA 4078), conduct focused neuro-behavioral/neuro-cognitive studies in rats, and attempt to assess ceramide/glucosylceramide levels in treated animals.

Comments:

- 1) It is recommended that requests for further studies be focused on the issues to be addressed by the study versus any specific technique/study design/species to be employed (i.e., time to onset of effect, reversibility/recovery of lesions or clinical signs, nature of the neurologic lesions [synaptic or axonal degeneration, cellular apoptosis, myelin degeneration, etc.], ceramide/glucosylceramide accumulation, etc.).
- 2) It is recommended that the sponsor be allowed to select a species other than rats for focused neuro-behavioral/neuro-cognitive testing. To date, limited tests of miglustat in juvenile rats have demonstrated alterations in neuro-behavioral performance, while tests in adult rats have demonstrated histologic alterations without concomitant behavioral signs. Thus, it is unclear that the rat is responding in a manner predictive of the human response or that it would be an effective test system for assessing the mechanism of the adverse drug action.

IV. Summary

A review of the action package for NDA 21-348, ZAVESCA® indicates that the product has been extensively evaluated in multiple acute, sub-chronic and chronic repeat-dose non-clinical safety studies (up to 12 months in rats and monkeys, one month in mice and dogs, genotoxicity and reproductive toxicology studies) to support potential approval for chronic use in the treatment of patients with Type 1 Gaucher Disease. However, it should be noted that carcinogenicity studies (typically required for pharmaceuticals that are likely to be used on a chronic basis) have not been completed (rat 2-year study) or initiated (mouse study) in accordance with an agreement made between the Div. and Sponsor in Aug. 1999. Follow-up studies to address concerns related to adverse neurologic signs noted in multiple patients and in non-clinical toxicology studies are recommended.

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/s/

David Morse
6/19/02 12:16:02 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-348

Review number: 1

Sequence number/date/type of submission: 000/August 16, 2001/Original NDA application.

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Oxford Glycosciences, The Forum, 86 Milton Park, Abingdon, Oxon, OX14 4RY, U.K.

Manufacturer for drug substance:

Reviewer name: John Colerangle

Division name: Division of Metabolic and Endocrine Drug Products (DMEDP).

HFD #: 510

Review completion date: April 8, 2002.

Drug:

Trade name: Zavesca

Generic name (list alphabetically): Miglustat, N-butyl-deoxynojirimycin.

Code name: OGT 918, SC48344, [OGT 924 also known as SC49483 (prodrug) is a perbutyrate derivative of OGT 918 used in some toxicity studies].

Chemical name: 1, 5 (Butylimino)-1,5-dideoxy-D-glucitol.

CAS registry number: 72599-27-0.

Mole file number: N/A.

Molecular formula/molecular weight: C₁₀H₂₁NO₄/219.28

Structure:

Relevant INDs/NDAs/DMFs: (OGS), IND 60,197 (OGS),

Drug class: An imino sugar (Glucosyltransferase inhibitor)

Indication: Treatment of Type 1 Gaucher Disease.

Clinical formulation:

Ingredient	Content
Miglustat (OGT 918) – active ingredient	100 mg
Sodium starch glycollate	
Povidone (K30)	
Magnesium stearate	
Capsule	

The proposed clinical dose is 100 mg TID or 300 mg/day.

Route of administration: Oral.

Proposed use: For the treatment of type 1 Gaucher disease.

Disclaimer: Some tabular and graphical information from sponsor's submission may have been reproduced in this review.

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Executive Summary

I. Recommendations

A. Recommendation on Approvability

Pharmacology/Toxicology recommends approval of this drug for the proposed indication.

B. Recommendation for Nonclinical Studies

The preclinical studies are adequate to support the safety of the 100 mg TID dose. A rat carcinogenicity study was accepted on 8/4/99 as a Phase IV commitment. ECAC reviewed the rat dose selection on 12/18/01.

C. Recommendations on Labeling

Draft +
Labeling

1 page(s) of
revised draft labeling
has been redacted
from this portion of
the review.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Acute, subacute, subchronic and chronic toxicology studies were conducted in mice, rats, dogs and monkeys to evaluate the safety of the drug. In addition, genetic toxicology, special toxicology and reproductive toxicology studies (rats and rabbits) were conducted to further evaluate the safety of the drug. The major target organs are the GI tract (dog, monkey, rat), testes (dog, rat, monkey), epididymis (rat), seminal vesicles (rat, monkey), liver (rat, monkey, dog, mouse), pancreas (rat, monkey, mouse), kidney (rat), bone marrow (rat, monkey), heart (monkey, rat), spleen, thymus and lymph nodes (rat, monkey, mouse), kidney (rat) and hematopoietic system (rat, dog, monkey).

The dominant feature of toxicity was profound diarrhea and its secondary effects on the GI tract especially in dogs and rats. Severity of the diarrhea increased with dose and sometimes progressed to bloody diarrhea or black tarry stool. In the chronic rat toxicology study, the frequency of diarrhea was high during the early treatment period but completely subsided with time. In the dog, necrosis of crypts of epithelium with dilatation and plugging, necrosis of villous tips occurred at $\geq 8X$ the therapeutic dose based on mg/m^2 . GI toxicity in rats occurred at $\geq 7X$ the therapeutic dose based on AUC. The GI lesions observed include increase in the mitotic figures in (cecal epithelium), cytoplasmic vacuolation of chief cells, ulcer, hyperkeratosis, inflammation, hemorrhage (stomach), dilatation of crypts, necrosis, edema, inflammation (colon), mucosal necrosis, inflammation, hemorrhage (cecum), depletion of goblet cells (intestines), villous atrophy (jejunum and ileum), and serosal fibrosis (esophagus).

While the Rhesus monkey showed GI toxicity similar to those observed in rats and dogs, the cynomolgus monkey had GI lesions in the gut mucosa at doses $\geq 6X$ the therapeutic dose (based on AUC) for 1 year with OGT 924. GI lesions in the rhesus monkey (ulcer, inflammation, necrosis and hemorrhage of the colon, cecum) were observed at $\geq 32X$ the therapeutic dose based on mg/m^2 . In the cynomolgus monkey, GI lesions were observed in the cecum, colon, stomach (pigmented macrophages, granulomatous inflammation) at doses $\geq 6X$ the therapeutic dose based on AUC.

Decreased mean body weight and/or body weight gain was observed across all species. Mean body weight was decreased by 12% (at doses $\geq 2X$ the therapeutic dose based on AUC) to 32% (at 10X the therapeutic dose based on AUC) in male rats following 13 to 52 weeks of treatment. Values for female rats range from 14% (at doses $\geq 7X$ the therapeutic dose based on AUC) to 26% (at 11X the therapeutic dose based on AUC). In mice, mean body weight was decreased by 12% (at 39X the therapeutic dose based on mg/m^2) and diarrhea occurred at $\geq 58X$ the therapeutic dose based on mg/m^2 following 2 weeks of treatment. Diarrhea occurred in all dogs treated with OGT 918 (at doses $\geq 2X$ the therapeutic dose based on mg/m^2) and to a negligible extent in dogs treated with OGT 924. This was the sponsor's basis for considering development of the pro-drug OGT 924 at one time. However this did not occur across species. Mean body weight decrement of 26% was observed at 89X the therapeutic dose based on mg/m^2 . Monkeys treated for 52 weeks with OGT 918 also experienced diarrhea at doses $\geq 11X$ the therapeutic dose based on AUC. Decreased mean body weight ranging from 18% to 32% for males (at doses $\geq 9X$ the therapeutic dose based on AUC) and 14% to 26% for females (at doses $\geq 7X$ the therapeutic dose based on AUC) was observed. The GI,

body weight and diarrhea effects appear to be related. The GI toxicity might have resulted in poor nutrient absorption, decreased body weight and/or body weight gain and the associated diarrhea. The diarrhea could also relate to the pharmacologic activity (inhibition of glucosidase - prevention of hydrolysis of disaccharides, potentially leading to osmotic diarrhea) of the drug. Based on the lymphoid depletion and associated spleen, thymus, and lymph node histopathology combined with alteration in lymphocyte trafficking, it is not surprising that significant inflammation is observed in the GI tract where gut associated lymphoid tissue (GALT) is present.

Clinical signs and histopathology findings suggestive of neurotoxicity were observed in the dog, rat and monkey. Ataxia, diminished/absent pupillary, palpebral or patellar reflexes were observed in the dog at doses $\geq 54X$ the therapeutic dose based on mg/m^2 . In addition, tremor and absent corneal reflexes were also observed at doses $\geq 11X$ the therapeutic dose based on mg/m^2 . However, there was no histopathology finding to support the clinical signs observed in the dog. Histopathology findings in monkey brain (vascular mineralization, mineralization and necrosis of white matter) and spinal cord (vascular mineralization) were observed at doses $\geq 4X$ the therapeutic dose based on AUC with no apparent clinical signs. Monkeys did not show signs of recovery. Vacuolation of the white matter of the brain was observed in both treated and control rats at doses $\geq 6X$ the therapeutic dose based on mg/m^2 . These findings were not dose-related but incidence increases with dose compared to control. Neurotoxicity has been observed at the therapeutic dose in clinical trials. A juvenile rat study in weanlings dosed post natal days 12 -70 given ≥ 20 $mg/kg/d$ had marked vacuolation of the brain and sciatic/tibial nerves. Both males and females exhibited head tilting. Development of learning, locomotor, auditory function, righting reflex and vision were unremarkable. Balanopreputial separation was delayed in males given 180 $mg/kg/d$ and delayed vaginal perforation occurred in females given ≥ 20 $mg/kg/d$.

Lymphoid/lymphocyte depletion was observed in the spleen, thymus, mesenteric lymph node, Peyer's patches of the ileum and submaxillary lymph node in rats dogs and mice. In rats, lymphocyte depletion occurred at doses $> 3X$ the therapeutic dose based on AUC and at $> 55X$ the therapeutic dose based on mg/m^2 following 4 weeks of treatment. Chronic studies in the rat revealed, lymphoid atrophy of the spleen, mesenteric lymph node and thymus at doses $\geq 4X$ the therapeutic dose based on AUC. Treatment up to 4 weeks in the dog caused thymic involution ($4X - mg/m^2$), decreased thymocytes, atrophy (lymphoid depletion) of the Peyer's patches of the ileum ($9X - mg/m^2$) and lymphoid depletion of the mesenteric lymph node ($\geq 4X - mg/m^2$). Thymic involution was observed in the mouse at doses $\geq 20X$ the therapeutic dose based on mg/m^2 . These effects may relate to a combination of the anti-HIV activity and pharmacologic activity of the drug. Since macrophages containing stored glucosylceramide are typically found in the bone marrow, liver and spleen, it is likely that the observed Lymphoid/lymphocyte depletion is also due to the pharmacologic activity of the drug. Lymphocyte subset analysis in peripheral blood, spleen and thymus of monkeys exposed for 4 weeks and rats exposed for 26 weeks revealed a lack of direct cytotoxic effects on lymphocytes. However in female monkeys a decrease in the number of splenic CD8+HLA-DR+ cells; an immature Tcell subset is observed which the sponsor attributes to an indirect effect of OGT 918 on thymus cell maturation. In treated rats and increase in CD4CD8 T cells in the blood and spleen were observed following OGT 924 exposure. This increase is attributed to a significant increase in the absolute number of CD3+CD4+ T cells in the blood and non-significant decrease in CD3+CD8+ T cells of the spleen. Together this indicates that

OGT 918 can alter lymphocyte trafficking. The lymphocytopenia observed in spleen, thymus, lymph nodes (mesenteric, submaxillary) and Peyer's patch of the ileum in multiple species provide histopathologic evidence of a drug related effect rather than simply redistribution, although direct lymphocyte cytotoxicity was not observed in monkeys and rat.

Bone marrow toxicity was observed in subacute rat studies and chronic monkey studies. In rats, bone marrow hypocellularity with fat replacement occurred at doses $\geq 3X$ the therapeutic dose based on AUC. Bone marrow hypocellularity with necrosis was also observed in the rats at $136X$ based on mg/m^2 . Chronic monkey studies also revealed bone marrow hypocellularity with fat replacement at $9X$ the therapeutic dose based on AUC. Bone marrow toxicity has been observed at the therapeutic dose in clinical trials. Since macrophages containing stored glucosylceramide are typically found in the bone marrow and other tissues, it is likely that the observed bone marrow toxicity is due to the pharmacologic activity of the drug.

Ceramide forms the backbone of all glycosphingolipids and has been associated with inducing apoptosis and some function as a second messenger. Analogues of OGT 918 have been shown to be cytotoxic at least in part due to accumulation of ceramide. The sponsor contends that OGT 918 allegedly does not cause toxic levels of ceramide to accumulate. However macrophages containing glucosylceramide are typically found in bone marrow. Although glucosylceramide levels were not measured in the nonclinical studies the bone marrow hypocellularity/necrosis present in rat and monkey suggest that this mechanism may be operational despite the sponsors claim. The erythrocyte parameter changes in multiple species further support this hypothesis. Interestingly the bone marrow hypocellularity with fatty replacement appeared predominately with chronic dosing whereas peripheral blood cell effects are seen within 4 weeks and continued into the chronic studies.

Decreased red blood cell parameters were observed in the mouse, rat, dog and monkeys. Hemoglobin, hematocrit and RBC count were all significantly decreased in the dog at $8x$ the therapeutic dose based on mg/m^2 . In rats, hemoglobin, hematocrit and RBC were also significantly decreased at doses: $\geq 10X$ the therapeutic dose (AUC, 4 weeks), $\geq 2X$ the therapeutic dose (AUC, 13 weeks), $\geq 5X$ the therapeutic dose (AUC, 26 weeks) and $\geq 8X$ the therapeutic dose (AUC, 52 weeks). Monkeys also showed decreased RBC, hematocrit and hemoglobin at $8X$ the therapeutic dose (AUC, 52 weeks). While similar effects were observed in the mouse and the decrements were dose-dependent, the difference between treated and control groups was not statistically significant. The decreased hematology parameters may be secondary to the bone marrow toxicity induced by the pharmacologic activity of the drug.

Cardiac toxicity characterized as degenerative cardiomyopathy was observed in rats at doses $\geq 22X$ the therapeutic dose based on AUC, following a 13-week study. This lesion was not observed at the end of the recovery period. In a 52-week study, degenerative cardiomyopathy was observed again in rats at doses $\geq 4X$ the therapeutic dose based on AUC. However, some control animals also had the lesion. Very little/no recovery occurred after a 4-week period. Acute inflammation of the heart was noted in monkeys found dead at doses $\geq 32X$ the therapeutic dose based on mg/m^2 after 4 weeks of treatment.

Chronic progressive nephropathy was observed in rats at 22X the therapeutic dose based on AUC, following a 13-week study. In 26-week and 52-week studies, the same lesion was observed at 4x and 3x the therapeutic dose based on AUC, respectively. Very little/no recovery occurred after a 4-week period.

Cataracts were observed in rats after 52-week studies at 4x the therapeutic dose based on AUC. Partial recovery was observed after a 4-week period. The cataracts may be related to the pharmacologic effects of the drug. The drug causes perturbations in lipid metabolism. Perturbations in lipid metabolism are associated with enhanced lipid peroxidation which generates oxygen free radicals. Oxygen free radicals are important known causes of tissue damage including cataracts.

Drug adverse effects were observed in the testes and epididymis of rats, seminal vesicles, sperm morphology and sperm parameters (concentration, motility) of rats and monkeys. 4-week studies in rats showed decreased spermatogenesis (testes), hypospermia (epididymis), degeneration of germinal epithelium (testes & epididymis) and atrophy (seminal vesicle) at $\geq 6X$ the therapeutic dose based on mg/m^2 . Following 13-week studies in rats, desquamated germ cells (testes & epididymis), seminiferous tubule atrophy (testes) were observed at doses $\geq 0.3X$ the therapeutic dose based on AUC. In another study of similar duration, atrophy and degeneration (testes) that increased in incidence and severity with dose was observed at doses $\geq 3X$ the therapeutic dose based on AUC and dystrophy (testes) was observed at 22X the therapeutic dose based on AUC. While safety margins based on AUC are small (0.3X to 5X), safety margins based on mg/m^2 ranged from 6X to 136X. In a 26-week rat study, testicular atrophy/degeneration that showed no recovery was observed at doses $\geq 3X$ the therapeutic dose based on AUC. Sperm motility, concentration and number of normal sperm were all decreased at doses $\geq 3X$ the therapeutic dose based on AUC and showed partial recovery. Chronic studies in rats (52 weeks) also showed hypospermia (epididymis), atrophy of seminiferous tubules, aspermatogenesis, edema, multinucleated giant cells and hyperplasia of interstitial cells (testes) at doses $\geq 5X$ the therapeutic dose based on AUC. These lesions showed little or no recovery after 4 weeks. A 52-week study in monkeys did not demonstrate any effects on the testes or epididymis. Mineralization of the seminal vesicle was observed at doses $\geq 4X$ the therapeutic dose based on AUC. Sperm concentration decreased, whereas number of amorphous sperms increased in a dose dependent manner. These effects also occurred at doses $\geq 4X$ the therapeutic dose based on AUC.

Female fertility and embryonic development studies were conducted in both rats and rabbits. In rats, doses $\geq 60 \text{ mg}/\text{kg}/\text{d}$ ($\geq 2X$ the therapeutic dose based on mg/m^2), decreased litter and fetal weight, increased post implantation loss as well as embryo-fetal deaths. Maternal toxicity (10% decrease in body weight, 29% decrease in body weight gain) occurred at 180 $\text{mg}/\text{kg}/\text{d}$. Visceral malformations (absent innominate artery, misshapen ventricle, lungs with reduced size) and skeletal malformations (wavy ribs, hemicentric thoracic vertebra) were observed at 180 $\text{mg}/\text{kg}/\text{d}$ (6X the therapeutic dose based on mg/m^2). NOAEL for rat fetal toxicity was 20 $\text{mg}/\text{kg}/\text{d}$ (0.6X the therapeutic dose based on mg/m^2). In rabbits, an increase in early embryo-fetal deaths and pre-implantation loss was observed at 45 $\text{mg}/\text{kg}/\text{d}$ (3X the therapeutic dose based on mg/m^2) whereas, runted fetuses were observed at doses $\geq 30 \text{ mg}/\text{kg}/\text{d}$ (2X the therapeutic dose based on mg/m^2). Maternal toxicity (decreased body weight gain) occurred at 15 and 45 $\text{mg}/\text{kg}/\text{d}$. Visceral malformations (aortic arches with additional blood vessel) increased in

a dose dependent manner achieving statistical significance at ≥ 15 mg/kg/d ($\geq 1X$ the therapeutic dose based on mg/m²). In a rabbit teratogenic study, doses ≥ 3 mg/kg/d ($\geq 0.2X$ the therapeutic dose based on mg/m²) caused an increase in pre-implantation loss whereas doses ≥ 10 mg/kg/d ($\geq 0.6X$ the therapeutic dose based on mg/m²) caused an increase in post-implantation loss. Maternal toxicity (decreased body weight gain) occurred at all doses. Rabbit fetal visceral malformations (missing brachiocephalic and left subclavian branching variation) were observed at 30 mg/kg/d (2X the therapeutic dose based on mg/m²) with incidences greater than those of historical control. NOEL for the rabbit developmental toxicity was 10 mg/kg/d (0.6X the therapeutic dose based on mg/m²). The results of the fertility tests indicate that Zavesca does not impair female fertility, but it does affect the morphology and motility of sperms.

The peri- and post-natal study in the rat did not show any harmful effect of Zavesca on the somatic and reproductive development of male and female F1 animals nor on their fertility or the development and survival of F2 rats. However, mean live birth index and mean survival index were statistically significantly decreased at doses ≥ 60 mg/kg/d ($\geq 2X$ the therapeutic dose based on mg/m²). A decrease in number of F1 pups born was observed at 180 mg/kg/d (6X the therapeutic dose based on mg/m²). Body weight gain of F1 pups decreased in a dose-dependent manner. At 6X the therapeutic dose, body weight gain was decreased by 18% and 17% for male and female F1 pups respectively. NOEL for the peri- and post natal study in rat was 20 mg/kg/d (0.6X the therapeutic dose based on mg/m²). Sponsor stated that transplacental transfer of Zavesca has been confirmed.

B. Pharmacologic Activity

Zavesca is an inhibitor of the enzyme glucosylceramide synthase, a glucosyl transferase enzyme responsible for the first step in the synthesis of most glycolipids. Glucosylceramide synthase catalyses the transfer of glucose from a UDP-glucose donor to a ceramide acceptor to form the product glucosylceramide (GlcCer) which is the building block of all glucosphingolipids. Glucosphingolipids play an important role in cell signalling, development, differentiation and host-pathogen interactions. They are important constituents of mammalian cells. Gaucher disease is caused by a failure to degrade glucosylceramide, resulting in the lysosomal storage of this material within tissue macrophages and widespread pathology. Glucosylceramide synthase is ubiquitous in tissues, hence this explains the multi-tissue nature of toxicity observed and relates to the pharmacologic activity of OGT 918.

C. Nonclinical Safety Issues Relevant to Clinical Use

The toxicity of OGT 918 relates to its mechanism of action. OGT 918 inhibits glucosylceramide synthase which transfers glucose to ceramide to form glucosylceramide; the building block of glucosphingolipids. Earlier deoxynorjirimycin analogues were associated with cytotoxicity due to ceramide accumulation in tissues. Ceramide is associated with inducing apoptosis and a role in signal transduction. The sponsor contends that OGT 918 does not induce cytotoxicity. This appears to be based on lymphocyte subset analysis which demonstrates a lack of cytotoxicity but demonstrated alteration in lymphocyte trafficking. Ceramide accumulation might explain the neurotoxicity seen in the clinic (tremor, paresthesia, numbness) and in animals (vascular mineralization of white matter and spine, necrosis/mineralization/vacuolation of white matter) in rat, dog and monkey at $<10X$ human therapeutic exposure. At

exposures 50X:human therapeutic exposure, ataxia, attenuated pupillary, palpebral or patellar reflexes were observed in the dog.

Likewise lymphoid depletion in spleen, lymph node, thymus, Peyer's patch of the ileum combined with the severe GI inflammation/necrosis (GALT) in multiple species at <10X human therapeutic exposure are suggestive of ceramide accumulation. The severe diarrhea (bloody, black tarry stool) may be associated with the GI toxicity and/or the ability of OGT 918 to inhibit α -glucosidase which would prevent the hydrolysis of disaccharides potentially leading to osmotic diarrhea in multiple species at < 10X human therapeutic exposure. In chronic toxicity studies the frequency of diarrhea was high early in treatment and subsided over time. This would also explain the body weight loss observed at this exposure.

In addition to toxicity possibly related to ceramide (glucosylceramide) accumulation there are toxicities which may be attributable to alterations in lipid metabolism. Cataracts were observed in rats at 4X therapeutic exposure. Perturbations in lipid metabolism can be associated with enhanced lipid peroxidation generating free radicals. Oxygen free radicals are associated with tissue damage and cataract formation. Similarly perturbations in lipid metabolism may be responsible for the fatty replacement and bone marrow hypocellularity at <10X therapeutic exposure in rat and monkey. Decreased erythrocyte parameters were observed in rodents, dog and monkey at \geq 10X human therapeutic exposure. A component of the neurologic and lymphoid toxicities noted previously may also involve perturbations in lipid metabolism. The sponsor has not performed mechanistic studies that address these possibilities.

Degenerative cardiomyopathy and chronic progressive nephropathy (not recoverable) was observed in rats at \geq 4X therapeutic exposure following chronic dosing. Acute myocardial inflammation was observed in monkeys found dead at \geq 100X human therapeutic exposure after 4 weeks of treatment. In a 1-year monkey study, increase in QRS complex by 20 msec was observed in a HD (2000 mg/kg/d) male. The HD corresponds to a C_{max} of 12 μ g/ml.

OGT 918 was associated with dystocia, delayed parturition and fetal death in rats at 2X human therapeutic exposures. Maternal toxicity was present in all doses examined in the rabbit.

A standard genotoxicity test battery consisting of: bacterial reverse mutation, chromosomal aberration in human lymphocytes, gene mutation in Chinese hamster ovary cells and in vivo mouse micronucleus demonstrates neither mutagenic nor clastogenic activity under the study conditions. Carcinogenicity testing was accepted as a Phase IV commitment on 8/4/99.