

## PHARMACOLOGY:

### **Primary pharmacodynamics:**

**Mechanism of action:** Growth hormone is produced and secreted by cells of the anterior pituitary gland. This hormone is essential in the promotion of skeletal and organ growth. The growth promoting action of GH is mediated by insulin-like growth factor 1 (IGF-1). When GH is present in excessive amounts in children, the clinical syndrome of gigantism is observed. However, when GH is produced in excessive amounts due to pituitary adenoma in adults acromegaly develops. Acromegalic patients exhibit coarse facial features, bony overgrowth, soft tissue swelling and hypertrophy of the internal organs. In acromegaly, both the circulating concentrations of GH and of IGF-1 (mediator of GH action) are increased dramatically. When B2036-PEG binds to the GH receptor, it is capable of displacing native GH. Therefore, **signal transduction is blocked and circulating concentrations of IGF-1 are decreased.** Reduction of IGF-1 concentration leads to amelioration of the signs and symptoms of acromegaly. Therefore, measurement of IGF-1 concentrations is a direct indication of the activity of B2036-PEG.

Human GH appears to bind to its receptor in a two step process. GH binds to one GH receptor at Site 1; this complex then binds to a second GH receptor at Site 2. B2036-PEG differs from GH in three ways. First, amino acid substitution at Site 1 insures binding avidity to GH receptor. Second, an amino acid substitution at Site 2 prevents binding to the second GH receptor. Third, PEGylation decreases the potential for immunogenicity (created by the amino acid substitutions) and increases the half life of the compound.

### Drug activity related to proposed indication:

B2036-PEG has been studied for its primary pharmacological effects in animals by measuring the changes both in GH and in IGF-1 concentrations. In addition, the species specificity of B2036-PEG binding to hepatic GH receptors has been determined. Further, the receptor specificity of B2036-PEG has been determined by measuring compound binding to a variety of receptors, including the prolactin receptor.

### **Study Title: Binding of B2036-PEG to liver growth hormone receptors (SEN-209):**

50 to twenty million hepatocytes or commercially prepared microsomal membranes from mouse, rat, rabbit, dog, rhesus monkey, and human were used to study the B2036-PEG binding to GH receptor. Microsomal membranes corresponding to 200 mg protein were incubated with <sup>125</sup>I-hGH and with various amounts of unlabeled B2036-PEG, ranging from \_\_\_\_\_ Radioactivity associated with the membrane pellets were measured using a gamma counter. Assays at each concentration were performed in triplicate.

**EC<sub>50</sub> Values For Displacement Assays**

<b>Species</b>	<b>EC<sub>50</sub> (nM)</b>	<b>95% Confidence Interval (nM)</b>
<b>Human</b>	—	<b>3.57-6.10</b>
<b>Monkey</b>	<b>1.42</b>	<b>0.77-2.61</b>
<b>Rabbit</b>	<b>2.04</b>	<b>0.55-7.61</b>
<b>Dog</b>	<b>39.8</b>	<b>14.1-112</b>
<b>Mouse</b>	<b>98.6</b>	<b>21.1-462</b>
<b>Rat</b>	<b>960</b>	<b>175-5270</b>

Based on the EC<sub>50</sub> values, **human, rhesus monkey, and rabbit receptors have similar binding constants for B2036-PEG.** Dog shows somewhat lower affinity while rodents, especially rat receptors, have very low affinity for B2036-PEG. The binding is estimated to be a few hundred-fold less for rodents than for humans, monkey and rabbits.

**Study Title: A study of the pharmacokinetics and pharmacodynamics of GH antagonists in mice (SEN-101, Not a GLP study)**

Several potential growth hormone antagonists (G120K-PEG, B2024-PEG, B2036-PEG) were administered to mice as single IP, IV or SC doses. Additionally, multiple SC dosages were administered at several concentrations. Serum concentrations of G120K-PEG were determined through 5 days after a single administration and after 5 daily administrations. Serum concentrations of B2024-PEG and B2036-PEG were determined after 5 daily administrations at dosages ranging from 0.25 to 2 or 4 mg/kg/day. IGF-1 concentrations were also determined. Livers were harvested from mice administered compounds for 5 days and analyzed for growth hormone receptor concentration. The C<sub>max</sub> value for B2036-PEG after 2 mg/kg/day was approximately 50 µg/ml and was reached on Day 4 after the first injection.

After multiple administration at 4 mg/kg/day of G120K-PEG and B2024-PEG, a significant, dose-dependent suppression of IGF-1 was evident on Day 3 with maximum suppression (up to 70%) of IGF-1 concentration achieved at a dose of 1 mg/kg on Day 6 (2 days after last injection). **G120K-PEG and B2024-PEG** were equally potent in lowering serum IGF-1 concentrations in mice. B2036-PEG administration at 2 mg/kg/day led to a maximal suppression of IGF-1 concentration of 30% on Day 5. In binding studies, both G120K-PEG and B2024-PEG demonstrated binding to mouse liver GH receptor while B2036-PEG did not bind to any great extent. **The lack of binding explains why B2036-PEG was not effective in lowering serum IGF-1 concentrations in mice.**

**Study Title: Action of growth hormone antagonists in mice: discussion of immunogenicity data (SEN-122)**

PEGylation of compounds are done to increase the residence time of the parent molecule in blood and to reduce immunogenicity. Mice (2 mice/compound) were given either G120K, B2034, B2036 or their pegylated analogs (G120k-PEG, B2034-PEG, B2036-PEG). Growth hormone antibodies in serum samples from mice transgenic for human growth hormone (hGH) were measured using a non-GLP non-specific (<sup>125</sup>I-hGH) RIA method. Serial dilutions of mouse sera were incubated with 0.05 M phosphate buffered saline and <sup>125</sup>I-hGH at 4°C for 16-18 hours. Antibody/antigen complexes were precipitated using goat anti-mouse Y globulin diluted 1:100 in 10% polyethylene glycol. Samples were centrifuged for 30 minutes at 4°C; supernatants were removed and the pellets were counted in a gamma counter.

Animals administered G120K, G120K-PEG did not develop a positive response, nor did mice given B2034-PEG. One of the to mice treated with B2034 developed a positive response. **Both mice administered B2036-PEG had weak positive responses with 18 to 32% specific binding on Day 35 with titers of 1:250 and 1:300. No antibody response to B2036 was observed.**

The ability of B2036 and B2036-PEG to produce antibodies was also measured in monkeys (Wilson, 1995). Since B2036 and B2036-PEG are derived from human growth hormone, antibody production was anticipated. Two male monkeys/treatment were given SC injections of B2036 or B2036-PEG daily for 14 days at 1 mg/injection (0.2 mg/kg/day). Serum was analyzed for antibody production on Days 1, 7, 14, 21, 28, 35 and 42. **One monkey from each treatment group had a mild antibody response with peak titers occurring on Day 28 at 1:220 (B2036-PEG) and 1:70 (B2036).** IGF-1 levels were not affected by B2036 administration. However, in animals given B2036-PEG, IGF-1 levels decreased from 200-400 ng/ml at baseline to ≤50 ng/mL by Day 7.

**Study Title: The human growth hormone antagonist B2036 does not interact with the prolactin receptor (SEN-210).**

Because human GH is able to bind to the human prolactin receptor (hPRLR), B2036-PEG, B2036, G120K-PEG and G120K were tested in several in vitro assays for their ability to interact with PRLR. In the first assay, rat Nb2 lymphoma cells, a cell line that proliferates when stimulated with lactogenic hormones, were incubated with the test compounds and cell density was determined

after three days of hormonal stimulation. In the second assay, a clone of human 293 fibroblasts was developed that expressed the PRLR. These cells were incubated with the compounds and their ability to activate or inhibit signaling mediated by PRLR was studied. In the second assay, compounds were examined for their ability to bind to the hPRLR using the HL5 cloned receptor. In all tests, neither B2036-PEG nor B2036 bound, activated or antagonized the PRLR from either rat or human origin, which demonstrates that the mutations within the B2036 binding Site 1 provide binding specificity towards the human GH receptor.

**Pharmacology summary:** B2036-PEG binds specifically to the GH receptor, thereby antagonizing the effects of GH. Hepatic GH receptors from monkey and rabbit demonstrate similar B2036-PEG binding as receptors from human liver while the binding to dog receptors is less. Mouse, and especially rat, demonstrate very little B2036-PEG binding to hepatic GH receptors.

When B2036-PEG was administered to mouse at dosages up to 1 mg/kg, no change in IGF-1 concentration was observed. At 2 mg/kg/day for 5 days, an approximately 30% decrease in IGF-1 was seen. In the rabbit, however, IGF-1 concentration fell 25% with a single dose of 3 mg/kg. After multiple doses to the rabbit, IGF-1 concentration decreased up to 80% below baseline. In pregnant rabbits, IGF-1 concentrations appear to decrease during gestation in the absence of any treatment. B2036-PEG administration at 10 mg/kg/day, but not lower, led to a further reduction in IGF-1. In addition, circulating GH concentration in these animals increased, suggesting an increased production of GH in response to absence of feedback inhibition.

In the rhesus monkey, IGF-1 and insulin like growth factor binding protein-3 (IGFBP-3) concentrations decreased after a single IV or SC dose of B2036-PEG at 0.3 or 1 mg/kg. The decrease could be seen 8 hours after compound administration and continued to decrease for 4 days, with suppression continuing through Day 7. Values returned to baseline by Day 14. The extent and duration of the response was dose-dependent. When compound was administered for 14 days at 0.2 mg/kg/day, a decrease in IGF-1 and IGFBP-3 was observed for up to 7 days after the last dose.

#### **SAFETY PHARMACOLOGY:**

Safety pharmacology studies were not conducted. Since growth hormone receptors in rodents have significantly lower affinity to B2036-PEG, pharmacological effect of B2036-PEG is relatively limited. Administration of B2036-PEG had little effect on IGF-1 in mouse and rat. Acute toxicity studies in animals found a significant SC injection site irritation. Similar findings have been noted with other pegylated compounds. The dermal irritability of B2036-PEG was examined in rabbits.

**Study Title: Dermal irritation potential of B2036-PEG after subcutaneous injection and effect of B2036-PEG on serum IGF-1 levels in the rabbit (SEN-115, Not a GLP study)**

B2036-PEG was administered to 3 female New Zealand white rabbits at 3 mg/kg as subcutaneous injections on Days 0, 1, 2, 6, 7 and 8 of the study. A control group (3 females) was administered vehicle (buffer for compound reconstitution) following the same schedule. Blood was collected from each rabbit pre-dose and at 2 and 6 hours after dose administration on study days 2, 3 and 8. Additional samples were taken at 26 and 30 hours after the last injection (2 and 6 hours on Day 9). Blood was processed to serum and stored frozen until analysis for IGF-1 concentrations. Control IGF-1 concentrations in rabbits were approximately 160 ng/mL. Serum IGF-1 concentrations decreased by 20 to 33% (=25%) in control animals after the first dose of vehicle. Mean serum IGF-1 concentrations of rabbits treated with B2036-PEG decreased to approximately 40 ng/mL at 24 hours after the first dose and approximately 30 ng/mL at 48 hours after the first dose (24 hours after the second dose) for an overall decrease of 75 to 80%. Both the control and compound-treated rabbits had approximately the same IGF-1 concentrations at 26 hours after the last dose administration as they did at 48 hours of the study. After the last dose administration,

serum IGF-1 concentrations returned to pre-study values in the control rabbits and had risen to 60 ng/mL in the B2036-PEG-treated animals by the last blood sampling time. Unlike mice, rabbits appear to respond to B2036-PEG which is not surprising considering the compound has been shown previously to bind to the rabbit GH receptor (SEN-209).

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**PHARMACOKINETICS/TOXICOKINETICS:**

**Individual Studies:**

**Study Title:** Subcutaneous or intravenous pharmacokinetic/pharmacodynamic study of B2036-PEG in mice (SEN-110)

B2036-PEG was administered to CD-1 mice as either a single intravenous (IV) dose of 0.3 mg/kg as a slow push or a single subcutaneous (SC) dose of 0.3 or 1 mg/kg. The vehicle used for this study was mannitol, glycine and phosphate buffer. Forty-two mice/sex were administered the IV dose, while each group of mice given B2036-PEG by the SC route contained 39 mice/sex. Blood samples were collected by cardiac puncture under anesthesia from 3 mice/sex/time point and processed to serum for analysis of B2036-PEG and IGF-1 concentrations. After IV administration, sampling occurred at predose and at 0.25 through 96 hours post-dose. After SC administration, sampling occurred at predose and 0.5 through 96 hours post-dose. Mice were sacrificed after blood collection.

Species		Dose, mg/kg	Route	Cmax, ng/ml	Tmax, hr	t 1/2β, hr	AUC, ng.hr/ml
Mice	M	0.3	IV	NA	NA	16.7±1.44	15,562 ±845
	F			NA		20 ± 1.9	137,816±9003
Mice	M	0.3	SC	198.8	12	NA	6,937 ±602
	F			2476	12	NA	87,661±6450
Mice	M	1	SC	710.72	12	NA	28,327±3262
	F			7974	12	NA	333,302±20755

B2036-PEG was well absorbed after SC injections. Female mice had higher exposure than males after both IV and SC route of administration. The bioavailability in males after 0.3 and 1mg/kg SC was 45 and 55% and in females 64 and 73%, respectively.

From pharmacodynamic standpoint, **there were no measurable changes in IGF-1 levels in mice following IV or SC administration of B2036-PEG.** This is not surprising since human GH does not interact with GH receptors in rodents and B2036-PEG is a modified human GH. The results of this study confirmed that B2036-PEG does not bind with significant affinity to the murine GH receptor and, hence, **does not exert a pharmacological effect in mice at the low doses studied.**

**Study Title:** Subcutaneous and intravenous crossover pharmacokinetic/pharmacodynamic study of B2036-PEG in rhesus monkeys (SEN-111)

B2036-PEG was administered to rhesus monkeys (3/sex) as a single bolus IV dose of 0.3 mg/kg in 5 mg/mL via the saphenous vein. After a 3-week period, the same animals received a SC dose (0.3 mg/kg). Following an additional 3-week period, the animals received a single administration of B2036-PEG SC at 1 mg/kg.

Species		Dose, mg/kg	Route	Cmax, ng/ml	Tmax, hr	t 1/2β, hr	AUC, ng.hr/ml
Monkeys	M	0.3	IV	NA	NA	27.5±.56	139,641±20,157
	F			NA	NA	31.1±2.9	145,784±11,289
Monkeys	M	0.3	SC	1481±174	20.6±3	26.1±4	98,285±9486
	F			1400±213	26.8±2	25.5±5.9	107,702±1,4015
Monkeys	M	1	SC	7286±608	32±4	NA	1,035,840±27469
	F			5327±782	40±4	NA	635,085±13,527

**There was no difference between males and females.** The terminal t 1/2 following 0.3 mg/kg SC dose administration was similar after iv and SC dosing (26 hours). Tmax was reached at 21 hours for males and 27 hours for females at 0.3 mg/kg SC and 32 hours for males and 40 hours for females at 1 mg/kg SC.

The volume of distribution after all of the dosages was similar, suggesting that while the high dose saturated the elimination processes, the unbound compound remained in the central compartment. **Bioavailability of the 0.3 mg/kg SC dose when compared to the IV dose was 81% and 70% for males and females, respectively, while bioavailability of the 1 mg/kg SC dose was essentially 100% for both sexes.**

Study Title: A subcutaneous pharmacokinetic study with B2036-PEG in rats (SEN-117)

B2036-PEG was administered SC to groups of SD rats (12/sex/group) at doses of 4, 40 or 160 mg/kg as a single dose. Blood samples were collected from 4 rats/sex/time from each dosage group from 10 minutes through 48 hours after compound administration. In the second part of the study, B2036-PEG was administered SC once daily for 10 consecutive days to 3 rats/sex/group. Dosages were 10 and 20 mg/kg/day. Blood was collected on Day 0 (first day of dosing), 1, 2 and 5 of dosing as well as on Day 10 through 15 (after the cessation of the study).

Following a single dose administration of B2036-PEG at 4, 40 and 160 mg/kg, C<sub>max</sub> and AUC values were higher for female rats than for male rats and both parameters increased with increasing doses in a dose-proportional manner. AUC<sub>0-24</sub> values were 192.0, 1838.0 and 5569.0 ng·hr/mL for males administered 4, 40 and 160 mg/kg, respectively and 316.0, 2684.0 and 11121.0 ng·hr/mL for females at these same dosages. **Mean IGF-1 concentrations were essentially unchanged in all groups from dose administration through 6 hours.** There was a decrease in IGF-1 from 782 ng/mL (immediately post-dose) to 582 ng/mL in males administered 160 mg/kg. For females, no change in IGF-1 concentration was observed at 4 mg/kg through 48 hours. At 40 and 160 mg/kg, there were decreases from 641 to 494 ng/mL and from 678 to 327 ng/mL for these groups, respectively. **The decrease in IGF-1 concentrations may reflect some pharmacological activity of B2036-PEG in the rat at these high dosages.**

The PK parameters after 10 consecutive daily administrations of B2036-PEG are shown in table below.

PK after repeated SC B2036-PEG		Dose, mg/kg	C <sub>max</sub> , µg/ml	V <sub>d</sub> , ml/kg	t <sub>1/2</sub> , hr	AUC, ng·hr/ml
Rats, SC	M	10	145 ± 9.7	17.8 ± 1.8	41 ± 6.8	33,088 ± 2,444
	F		161 ± 44	15.7 ± 2.1	38 ± 2.6	35,167 ± 6,114
Rats, SC	M	20	293 ± 2.6	13.2 ± 2.5	30 ± 6.6	65,336 ± 2,106
	F		366 ± 133	9.87 ± 4.3	26 ± 11	75,957 ± 21,951

C<sub>max</sub> and AUC values increased in a dose proportional manner after both single and repeated dosages. **Serum concentrations of B2036-PEG were generally higher for female than for male rats,** although this finding was more prominent after single than multiple administrations. In addition, the decrease in IGF-1 concentrations at ≥ 40 mg/kg was greater in females than males, suggesting that there may be some pharmacological activity at very large doses. The terminal elimination t<sub>1/2</sub> appeared to be dosage-dependent and decreased with increasing dosage, possibly reflecting the large volume of distribution at the lower dose.

Study Title: Tissue distribution and excretion of <sup>125</sup>I-B2036-PEG following a single SC administration of a mixture of <sup>125</sup>I-B2036-PEG and B2036-PEG to rats (SEN-121)  
 B2036-PEG/[<sup>125</sup>I]-B2036-PEG was administered to SD rats (24/sex) by SC injection as a single 3 mg/kg dose. Blood and selected tissues were collected and analyzed for total radioactivity. C<sub>max</sub> values for total radioactivity in the serum and blood cellular fraction were reached at 24 hours and then declined slowly. The ratio of radioactivity in the blood cell fraction to serum was 0.898 at 0.5 hours after dose administration and 0.333 at 24 hours.

The apparent maximum mean tissue concentration of radioactivity was reached in all tissues at 24 hours after dose administration with the exception of brown fat (injection site), stomach, and thyroid/parathyroid gland where the maximum was reached at 8, 8 and 72 hours, respectively. The highest radioactivity concentrations were found in brown fat (injection site) > serum > thyroid /parathyroid > ovaries, lungs and kidneys. Male rat tissue concentrations were somewhat lower than female tissue concentrations. Tissues containing the highest percentage of administered dose were muscle (3.74%), brown fat (injection site, 2.64%), liver (2.15%) and bone (1.24%). All other tissues each contained less than 1% of the administered dose. Whole body autoradiography confirmed the above findings and also demonstrated that radioactivity did not cross the blood-brain barrier.

In total, recovery of administered radioactivity in urine, feces and cage washes was 88.3% and 92.2% for males and females, respectively. It appears that the radioactivity associated with <sup>125</sup>I-B2036-PEG was absorbed slowly with Tmax reached at 24 hours. Distribution of radioactivity into tissues was minimal and radioactivity was not entrapped in any tissue. In general, tissues from female rats had slightly higher radioactivity concentrations than in tissues from males.

Renal excretion was the primary route of elimination of radioactivity from the rat, with more than 85% of excreted radioactivity found in the urine. Total recovery through 168 hours after dose administration was approximately 90%. Radioactivity levels in different tissues at 8 and 24 hrs postdose are shown in table below:

Mean Concentration of Radioactivity in Cellular Fraction, Serum, and Tissues at Specified Times After Administration of a Single Subcutaneous Dose of a Mixture of [<sup>125</sup>I]-B2036-PEG and B2036-PEG (3 mg equivalents of B2036/kg) to Rats (Group 1)

Tissue	µg Equivalents of B2036/g											
	8 Hours						24 Hours					
	Male		Female		Overall		Male		Female		Overall	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	0.410	0.237	0.574	0.071	0.492	0.181	1.03	0.090	1.32	0.237	1.18	0.229
Bone (femur)	0.374	0.040	0.351	0.023	0.363	0.032	0.706	0.047	0.612	0.064	0.659	0.072
Bone marrow (femur)	1.28	0.988	1.01	0.130	1.14	0.649	1.40	0.213	1.70	0.516	1.35	0.308
Brain	0.066	0.003	0.100	0.021	0.087	0.027	0.158	0.028	0.172	0.031	0.165	0.027
Cellular Fraction	2.57	1.37	2.87	0.957	2.72	1.07	4.72	0.487	5.94	0.956	5.33	0.951
Epididymus (testes)	0.413	0.059	NA	NA	0.413	0.059	1.08	0.162	NA	NA	1.08	0.162
Eyes (both)	0.220	0.004	0.259	0.033	0.240	0.030	0.433	0.029	0.684	0.068	0.599	0.146
Fat (abdominal)	0.113	0.026	0.147	0.011	0.130	0.026	0.446	0.064	0.302	0.052	0.374	0.094
Fat (brown)	147	10.4	63.2	31.8	105	50.5	19.5	4.45	15.4	3.75	17.4	5.10
Heart	0.513	0.053	0.703	0.093	0.608	0.124	1.56	0.179	1.90	0.171	1.73	0.241
Kidneys	0.897	0.172	1.19	0.171	1.04	0.223	1.78	0.176	2.25	0.394	2.02	0.374
Large intestine	0.292	0.030	0.290	0.015	0.291	0.021	0.619	0.105	0.784	0.100	0.702	0.129
Large intestinal contents and wash	0.074	0.005	0.089	0.011	0.082	0.011	0.068	0.018	0.078	0.002	0.073	0.013
Liver	0.592	0.086	0.856	0.119	0.724	0.171	1.21	0.109	1.58	0.428	1.39	0.345
Lungs	0.742	0.108	1.03	0.017	0.887	0.170	1.76	0.261	2.42	0.483	2.09	0.503
Muscle (thoracic, thigh)	0.136	0.027	0.128	0.042	0.132	0.032	0.230	0.062	0.233	0.036	0.241	0.046
Ovaries (female)	NA	NA	1.18	0.224	1.18	0.224	NA	NA	2.93	0.944	2.93	0.944
Pancreas	0.679	0.129	0.378	0.021	0.427	0.109	0.750	0.035	0.832	0.094	0.791	0.078
Prostate (male)	0.463	0.156	NA	NA	0.463	0.156	0.624	0.077	NA	NA	0.624	0.077
Serum	4.30	0.249	6.80	1.31	5.53	1.61	13.9	1.56	18.2	3.16	16.1	3.23
Small intestine	0.423	0.061	0.465	0.091	0.444	0.073	0.546	0.082	0.582	0.044	0.564	0.082
Small intestinal contents and wash	0.227	0.011	0.130	0.026	0.178	0.064	0.124	0.030	0.072	0.014	0.098	0.036
Spleen	0.369	0.029	0.462	0.016	0.415	0.025	0.639	0.054	0.796	0.107	0.717	0.115
Stomach	1.73	0.782	1.87	0.662	1.78	0.649	1.06	0.058	1.04	0.102	1.05	0.076
Testes (male)	0.454	0.033	NA	NA	0.454	0.033	1.21	0.095	NA	NA	1.21	0.095
Thyroid/Parathyroid	1.74	0.436	2.62	0.740	2.18	0.727	7.88	4.08	6.40	2.28	7.14	3.04

NA Not applicable.  
SD Standard deviation.

#### PK parameters:

Summary of pharmacokinetic parameters after single, multiple and steady state dosing regimen in different species are shown in table below:

**PHARMACOKINETIC PARAMETERS AT STEADY IN ANIMALS RECEIVING MULTIPLE DOSES OF B2036-PEG BY THE SUBCUTANEOUS ROUTE**

Study Number	Species	Dose (mg/kg)	Dosing Duration (Weeks)	C <sub>max</sub> (µg/mL)		AUC <sub>0-24</sub> (mg·hr/L)	
				M	F	M	F
SEN-107	Mouse	0.1	2	0.667		NC	
		3	2	8.24		NC	
SEN-117	Rat	10	10 days	145 / 161		33.1 / 65.3	
		20	10 days	293 / 366		35.3 / 76.0	
SEN-118	Rat	3	13	43.4 / 73.9		NC	
		10	13	166 / 166		NC	
		30	13	662 / 422		NC	
SEN-116	Rabbit	0.3	1	2.06		NC	
		1	1	11.5		NC	
		3	1	100		NC	
		10	1	424		NC	
SEN-108	Monkey	0.1	4	0.8080 / 0.626		NC	
		3	4	128.2 / 111.6		NC	
SEN-109	Monkey	0.3	26	1.66 / 1.30		0.116 / 0.100	
		1	26	9.31 / 6.54		0.763 / 0.569	
		3	26	34.9 / 34.2		2.83 / 2.83	

NC = Not calculated

**Absorption, Distribution and Excretion:**

B2036-PEG was well-absorbed after SC administration. The absolute bioavailability of a single SC dose in mice was approximately 50% for males and 70% for females and appeared to be somewhat greater at a higher dose. In rhesus monkey, the absolute bioavailability of a single SC dose was 70 to 81% after a 0.3 mg/kg dose and essentially 100% after a 1 mg/kg dose. The volume of distribution of B2036-PEG in the rat was approximately 15 mL/kg after 10 mg/kg/day SC and 20 mL/kg after 20 mg/kg/day SC. Clearance of B2036-PEG was approximately 0.3 mL/hr/kg in the rat for both sexes at both dosages.

After IV administration of B2036-PEG to mice and monkeys, serum concentrations declined following a bi-exponential. Feces accounted for less than 2% of administered radioactivity. Whether the urinary radioactivity was bound to B2036-PEG at excretion or, more likely, represents free iodine, has not yet been determined. Pharmacokinetic parameters of single subcutaneous dose pegvisomant in several species of animals are shown in table below:

Species		Dose, mg/kg	C <sub>max</sub> , ng/ml	T <sub>max</sub> , hr	t <sub>1/2β</sub> , hr	AUC <sub>0-n</sub> , µg·hr/ml (mg·hr/L)
Mice, SC	M	0.3 mg/kg	198.8	12	NA	6.9 ± 6.0
	F		2476	12	NA	87.7 ± 6.4
Mice, SC	M	1 mg/kg	710.72	12	NA	28.3 ± 3.3
	F		7974	12	NA	333.3 ± 20.7
Rats, SC	M	10 mg/kg	145 ± 9.7		41 ± 6.8	33.1 ± 2.4
	F		161 ± 44		38 ± 2.6	35.2 ± 6.1
Rats, SC	M	20 mg/kg	293 ± 2.6		30 ± 6.6	65.3 ± 2.1
	F		366 ± 133		26 ± 11	75.9 ± 21.9
Monkeys	M	0.3 mg/kg	1481 ± 174	20.6 ± 3	26.1 ± 4	98.3 ± 9.5
	F		1400 ± 213	26.8 ± 2	25.5 ± 5.9	107.7 ± 1.4
Monkeys, SC	M	1 mg/kg	7286 ± 608	32 ± 4	NA	1,035.8 ± 27.5
	F		5327 ± 782	40 ± 4	NA	635.0 ± 13.5

**Pharmacokinetics summary table for B2036-PEG in humans:**

Pharmacokinetics of Single Doses of Pegvisomant in Healthy Volunteers (Study SEN-3623)		
Parameter	Dose	
	20 mg, SC	10 mg, IV
$C_{max}$ (ng/mL)	1387.2 ± 628.7	4270.9 ± 624.8
$T_{max}$ (hr)	49.02 ± 15.93	6.45 ± 0.56
$AUC_{0-\infty}$ (µg-hr/mL)	207.8 ± 89.5	183.3 ± 79.9
$t_{1/2}$ (hr)	138.4 ± 68.3	138.0 ± 35.9
CL/F (mL/min)	1.80 ± 0.53	1.03 ± 0.34
V/F (L)	23.3 ± 18.0	12.4 ± 5.4
$F_e$	0.0015 ± 0.0015	0.0041 ± 0.0030

Data shown are mean ± SD for 12 subjects per dose group (crossover design).

$C_{max}$ , maximum serum concentration;  $T_{max}$ , time to maximum serum concentration;  $AUC_{0-\infty}$ , area under the serum concentration-time curve from time 0 to infinity;  $t_{1/2}$ , elimination half-life; CL/F, total body clearance (F, bioavailability and assumed to equal 1 in these calculations);  $F_e$ , fraction excreted into the urine from time 0 to 96 hours.

**Excretion:**

After IV administration of B2036-PEG to mice, serum concentrations declined following a bi-exponential equation with an  $\alpha$  phase  $t_{1/2}$  of less than 1 hour and a terminal elimination  $t_{1/2}$  of 17 to 20 hours. The terminal elimination  $t_{1/2}$  was approximately 17 hours after SC administration. In rats, elimination followed first-order elimination kinetics with terminal  $t_{1/2}$  values of 39 and 28 hours for 10 and 20 mg/kg/day dosages, respectively. In monkeys, serum concentrations also declined following a bi-exponential equation with an  $\alpha$  phase  $t_{1/2}$  value of 3.4 hours after IV administration. Terminal  $t_{1/2}$  values for both sexes were 26 and 30 hours after 0.3 mg/kg SC and IV doses, respectively. There was some evidence of non-linear elimination after a 1 mg/kg dose. Radioactivity ( $^{125}I$ ) associated with B2036-PEG was excreted primarily in the urine of rats, with approximately 85% of administered radioactivity in the 0 to 48 hour urine. Feces accounted for less than 2% of administered radioactivity. Whether the urinary radioactivity was bound to B2036-PEG at excretion or, more likely, represents free iodine, has not yet been determined.

**Metabolism:**

Specific metabolism studies with B2036-PEG have not been performed. It is expected that the non-pegylated portions of the molecule will undergo typical protein catabolism. Whether small peptide fragments with PEG attached will be further metabolized or excreted intact has yet to be determined. Since the PEG metabolism via alcohol dehydrogenase may lead to toxic metabolites to kidney future monitoring of renal parameters are warranted specially in patients with reduced renal functional reserve capacity.

**PK/TK summary:**

- After single dose administration of B2036-PEG to mice, but not monkeys, there was a difference between pharmacokinetic parameters for males and females.
- B2036-PEG was eliminated from mice and monkeys with a terminal elimination half-life of approximately 20 to 30 hours. Elimination in the monkey was saturable with 0.3 mg/kg doses eliminated following first order kinetics, while a dose of 1 mg/kg followed Michaelis-Menton kinetics.
- The volume of distribution of B2036-PEG was small for both mouse and monkey, suggesting that the drug remained in the central compartment.
- Bioavailability of B2036-PEG by the subcutaneous route was greater than 70% in the monkey and somewhat lower in the mouse.

**PK/TK conclusions:** Male rats had lower plasma concentrations than females. Elimination half-life of B2036-PEG is relatively long in animals (17-30 hrs). Since protein part of the B2036-PEG may become degraded similar to other protein, no serious toxicity from metabolism is suspected and lack of specific metabolism studies are less concerning than pegylated portion of B2036-PEG. After degradation of PEG from protein, PEG may go through metabolic pathway similar to ethylene glycol. Therefore, under chronic conditions, daily injections of B2036-PEG may become toxic in subjects with compromised renal function.

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## TOXICOLOGY:

**Study title:** A 6-month subcutaneous toxicity study of B2036-PEG in rats

**Key study findings:** Nephropathy in female rats 30 mg/kg/d (15 X human exposure), inflammation at injection site, hypertrophy of liver and kidney and decreased body weight and food consumption at 10 and 30 mg/kg/d (5 and 10 X human exposure, respectively). The NOAEL in female and male rats were 3 and 10 mg/kg/d, approximately 1.5 and 5 times the human exposure, respectively.

**Study no:** SEN-118 (lab study # \_\_\_\_\_)

**Volume #, and page #:** 1-5 and pages 1-1611

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** October 23, 1998 /completed on May 31, 2000

**GLP compliance:** yes

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** 3 Lot 1812001-B, 1812003-B, 1812004-B. The purity was assumed to be 100% (protein). The drug was obtained from \_\_\_\_\_

**Formulation/vehicle:** human growth hormone antagonist, 10 mg/ml. The drug compound was prepared in control vehicle and sterile water for injections. The control vehicle (3 ml/kg) contained 18 g/l mannitol, 0.68 g/l glycine and 5 mM sodium phosphate monobasic.

**Methods (unique aspects):** The toxicity of B2036-PEG was evaluated in rats after SC injections at interim 13 weeks (10/sex/dose) and at the end of the study, week 26 (15 /sex/dose). Some animals (4 to 5/sex/dose) were allowed a 4-week recovery period (week 30).

### Dosing:

**Species/strain:** Crl:CD®(SD)BR rat

**#/sex/group or time point (main study):** A total of 30 rats/sex/group was used at the start of the study. 10/sex/dose were terminated at the interim 13-week evaluation. 15/sex/dose were sacrificed at week 26. The 4 to 5 remaining animals per sex/dose were sacrificed after a 4-week recovery period.

**Satellite groups used for toxicokinetics or recovery:** 12 animals/sex/group for TK.

**Age:** 6 weeks

**Weight:** 178-251 for males, 144 to 195 for females

**Doses in administered units:** 0, 3, 10 and 30 mg/kg. Drug solution was prepared daily and samples were taken for future analysis. The drug levels in the administered solutions were equal to or slightly higher than the target solution concentrations.

**Route, form, volume:** subcutaneous, solution, 3 ml/kg

### Observations and times:

**Clinical signs:** Twice a day

**Body weights:** weekly

**Food consumption:** Weekly

**Ophthalmoscopy:** Prior to and on Week 12 and 25

**EKG:** NA

**Hematology:** Standard Hematology test, WK 13, 26: Leukocyte, Erythrocyte, Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet, Prothrombin Time, Activated

Partial Thromboplastin Time (APTT), Differential Leukocyte Count Percent and Absolute Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil, Platelet, Red Cell Morphology.

Clinical chemistry: Standard tests, WK 13, 26: Albumin, Total Protein, Globulin, A/G Ratio, Total Bilirubin, Urea Nitrogen, Creatinine, Alkaline Phosphatase, Alanine Aminotransferase, Aspartate Aminotransferase, Gamma Glutamyltransferase, Glucose, Total Cholesterol, Calcium, Chloride, Phosphorus, Potassium, Sodium, Triglycerides

Urinalysis: Specific Gravity, pH, Urobilinogen, Total Volume, Color, Appearance, Protein, Glucose, Ketones (KET), Bilirubin (BIL), Occult Blood (BLD), Leukocytes (LEU), Nitrites (NIT)  
Microscopy of Sediment.

Gross pathology: Standard path, See addendum page 29

Organs weighed: See addendum for list page 29

Histopathology: All animals in the main study were examined. Animals were euthanized with CO<sub>2</sub> Standard tissue histopath, see addendum for list, page 29

Toxicokinetics: Blood samples (approximately 0.5 ml via lateral tail vein, 3 rats/sex/dose) for test article serum level determination were collected on study days 0, 90 and 178 just prior to dosing and 0.5, 1, 2, 4 and 8 hours after dosing. Serum was frozen until shipped on dry ice to \_\_\_\_\_, for analysis.

Other: In the initial analysis of plasma concentration of anti B2036-PEG antibody was not validated properly. The results were considered invalid. No data was submitted or discussed in this submission. After reconstitution of the drug substance, samples were taken for validation of target solution concentrations. The measured concentrations were 1.3, 5.0 and 11.2 vs. target concentrations of 1.0, 3.3 and 10.0 mg/ml, respectively.

**Results:**

Mortality: Several animals died but the death were probably not drug related.

- One LD male died on Day 39 (brain hemorrhage)
- One LD male died on Day 174 (no findings)
- One LD female died on Day 147 (malignant lymphoma)
- One control male and female died on Days 78 and 45 ( urinary tract problems)

Clinical signs:

- Thickening at the injection site increased dose-dependently. Four males in HD recovery also had thickened skin.
- One MD male had firm moveable mass (preputial gland, chronic active inflammation) in urogenital area during recovery.

Body weights:

- Body weight and body weight gains in males were lower at several points during the course of monitoring. HD males had significantly lower body weight at Week 13 (-9.5%) and Week 26 (-15%).
- No significant change in body weight of female rats.

Body weight changes, From Baseline (WK 0)	Week	30 mg/kg/d	
		M	F
Body weight gain	13	-16.7%	-11%
changes, % decrease	26	-17.5%	

Food consumption: Food consumption of HD males was reduced at several time points by as much as 18% during week 17. No significant difference at Wk 26.

Ophthalmoscopy: No significant treatment related finding. However, nearly half of the males (=15/30) from all groups had bilateral corneal crystals prior to treatment.

Hematology:

Hematology		3 mg/kg/d		10 mg/kg/d		30 mg/kg/d	
		M	F	M	F	M	F
	Week						
Hemoglobin, % Δ	26					6.7	-4
Hematocrit, % Δ	26			5.6		6.9	
MCH, %Δ	26	4.6		8		6.8	
Platelet, %Δ	26			-10.7		-11.6	
Protime, % Δ	26					-7.4	
White cells, % Δ	13					-23	
	26					-21	
Lymphocytes, % Δ	13					-34	

Clinical chemistry: The decrease in alkaline phosphatase levels returned to normal levels after recovery period. The significance of lower alkaline phosphatase in treated animals is not clear. Although the source of ALP was not determined, the decrease in ALP could be due to decreased osteoclast/osteoblast activity.

Percent change relative to controls		3 mg/kg/d		10 mg/kg/d		30 mg/kg/d	
		M	F	M	F	M	F
	WK						
Albumin	13				14	19	14
	26					17.5	
Globulin	26			-14.3		-14.3	17.4
A/G ratio	13					36	
	26			39		46	
Protein	13						12
	26		8		5.3		
Alkaline phosphatase	13	-22		-38		-56%	-33%
	26			-27		-45%	-32%

Urinalysis: Urinary protein levels increased in females and males treated with B2036-PEG. Animals in HD group had moderate to abundant levels of urine protein suggesting nephropathy. Histopathology findings correlates with the urinary protein findings. The sponsor suggestion that the increase in urine protein is due to proteinaceous injection is totally incorrect. Proteinuria persisted during recovery, suggesting the drug-induced renal damage persisted demonstrated by excessive leakage of protein into urine. Since urinary protein was measured by a qualitative protein stick, presence of protein in urine ≥ 2 (slight to moderate) are presented in table below. The data was not analyzed. Sponsor could have applied a non-parametric ranking method to compare treatments.

Urinary protein		control		3 mg/kg/d		10 mg/kg/d		30 mg/kg/d	
		M	F	M	F	M	F	M	F
Urine Protein Scores	WK13 (n=10)	(3/10) 3,3,3	(0/10)	(4/10) 2, 3,3,3	(2/10) 3,3	(5/10) 2,2 3,3,3	(3/10) 2 3,3	(6/10) 2 3,3,3,3,3	(6/10) 2,2,2 3,3,3
	WK 26 (n=14-15)	(7/15) 2,2,2,2 3,3,3	(4/15) 2,2,2 3	(7/14) 2,2, 3,3,3, 3,3	(7/15) 2,2,2,2 3,3,3	(6/15) 3,3,3,3, 3,3	(11/15) 2,2, 3,3,3,3,3, 3,3,3,3,3	(8/15) 2,2,2, 3,3,3,3,3	(12/15) 2,2 3,3,3,3,3, 3,3,3,3,3
	Recov. (n=4-5)	(3/4) 2 3,3	(1/4) 2	(3/4) 2 3,3	(0/4)	(5/5) 2 3,3,3,3	(5/5) 2 3,3,3,3	(3/5) 3,3,3	(5/5) 2 3,3,3,3

Urine protein score of 2= slight to moderate, 3= moderate to abundant

**Organ weights:**

- Adrenal gland weight increased in male rats dose-dependently with no change in females.
- Liver and kidney weights increased in HD females by as much as 20% at the end of the study. The increase in kidney weight correlates well with nephropathy.

Absolute Organ weights that were significantly different from control	Week	3 mg/kg/d		10 mg/kg/d		30 mg/kg/d	
		M	F	M	F	M	F
Spleen, % Δ	13					-17.7	
	26					-19	
Adrenal, % Δ	13			34		41.5	
	26	20		18		39	
Liver, % Δ	13						16
	26					-19	20.6
Kidney, % Δ	26						18

Relative Organ to body weight	Week	3 mg/kg/d		10 mg/kg/d		30 mg/kg/d	
		M	F	M	F	M	F
Adrenal, % Δ	13			33.3		50	
	26	33.3		33.3		66.6	
Liver, % Δ	13				13		26
	26		10.6		12.8		24
Testes, % Δ	26					16.4	
Kidney, % Δ	13						11.5
	26						21.5

**Gross pathology:**

- One control male that had died before the end of the study was noted to have pelvic dilation in the kidney.
- Cyst in spleen of LD (2/14), MD (3/15), HD (3/15) males and LD female (1/15) was noted.
- Thickened injection site in LD male (3/14), MD (13/15), HD (15/15) males and HD (9/15) female

**Histopathology:**

Summary pathology of animals with early death: One LD male that died on Day 39, had submeningeal hemorrhage. Another female on LD group died on Day 147 with malignant lymphoma with metastatic lesion in the brain. Two controls that died early had urinary calculi and microscopic findings of transitional cell hyperplasia of the urinary bladder and ureter. Minimal degree of nephropathy with hydronephrosis was noted in one the control females. Thickening of the injection site was dose dependent and drug related.

**Histopathology Findings in Main Study:**

**Kidney: WK 13:** Kidney: A small increase in the frequency of renal tubular dilatation (1/20 C, 0/20 LD, 3/20 HD) and nephropathy (4/20 C, 8/20 LD, 10/20 MD, 5/20 HD) was observed in treated rats.

**WK 26:** Incidences of tubular dilatation (1/30 C, 1/30 LD, 6/30 MD, 5/30 HD) and tubular nephropathy (12/30 C, 17/30 LD, 16/30 MD, 16/30 HD) were increased in MD, HD rats. The nephropathy findings increased with treatment in females (2 C, 8 LD, 6 MD, 8 HD) but did not display clear drug-related incidence in males.

**Liver: WK13:** cytoplasmic vacuolization of hepatocytes was observed more frequently in HD rats (2/20 C, 2/20 LD, 1/20 MD, 6/20 HD).

WK26: Frequency and severity of hepatocytes vacuolation was increased and displayed a dose-related incidence and severity in all dose groups after 26 weeks of dosing (2/20C, 8/20 LD, 4/20 MD, 10/20 HD).

Lymph Node: Increase in vacuolated macrophage were observed in the submandibular and mesenteric lymph nodes in both HD male and female rats.

Injection Site: Dose-related inflammation and necrosis were observed.

Interim histopath findings in rats treated with B2036-PEG at Week 13:

Tissue	Sex	Dose, mg/kg/day			
		0	3	10	30
Adrenal gland	M	4/10	0	0	2/10
Vacuolation, cytoplasmic, minimal	F	1/10 (mild)	-	0	3/10
	M	-	-	-	-
Hypertrophy, cortical, minimal	F	1/10	-	1/10	0
Kidney,	M	3/10	5/10	7/10	4/10
Nephropathy, minimal	F	1/10	3/10	3/10	1/10
	M	0	0	0	1/10 (mod)
inflammation, suppurative, minimal	F	3/10	3/10	4/10	4/10
	M	0	0	1/10	1/10
Tubular Dilatation, minimal	F	1/10	0	2/10	3/10
Ureter	M	0	-	-	1/10
Hyperplasia, transitional cells, minimal	F	0	-	-	0
	M	0	-	-	1/10
mild	F	0	-	-	0
Liver,	M	0	1/10	0	2/10
Vacuolation, cytoplasmic, minimal	F	2/10	1/10	1/10	4/10
Lymph node, mesenteric	M	4/10	-	-	5/10
Dilatation, sinuses, minimal(+)	F	2/10	-	-	3/10
	M	3/10	-	-	1/10
moderate (++)	F	-	-	-	-
Vacuolated macrophages, mild	F	0	-	-	1/10
Lymph node, Sub	M	0	0	3/10	6/10
Vacuolated macrophage, minimal(+)	F	0	0	4/10	5/10
	M	0	-	-	2/10
moderate (++)	F	0	0	1/10	0
	M	-	-	-	-
plasmacytosis, medullary, minimal	F	2/10	4/10	4/10	8/10
	M	0	-	-	-
mild	F	5/10	3/10	3/10	2/10
Ovaries Atrophy, mild	F	0	-	-	1/10
Cyst, follicular, minimal	F	0	-	-	1/10
Injection site	M	4/10	3/10	9/10	1/10
Inflammation, chronic active, minimal	F	3/10	7/10	8/10	2/10
	M	2/10	5/10	1/10	6/10
Mild	F	0	0	0	1/10
	M	0	0	0	3/10
Moderate	F	0	0	0	1/10
Vacuolated macrophage, hypodermis,	M	0	3/10	1/10	7/10
Minimal	F	0	3/10	3/10	1/10
	M	0	3/10	5/10	3/10
Mild	F	0	2/10	3/10	8/10
	M	0	4/10	4/10	0
Moderate	F	0	1/10	3/10	0

Histopath findings in rats treated with B2036-PEG at Week 26:

Tissue	Sex	Dose, mg/kg/day			
		0 (15 M,F)	3 (14 M,15F)	10 (15 M,F)	30 (15 M,F)
Adrenal gland Vacuolation, cytoplasmic	M	2 min	-	-	1min
	F	0/10	-	-	3min
Harderian Gland, alteration	M	3 min	1 mil	-	1mil,1mod
Liver, Vacuolation, cytoplasmic, Vacuol. macrophage, peribiliary	M	1mil	3 mil,1mod	3min	4min,1mod
	F	1 min	4 min	1min	5min,1mil
	M	0	1/14min	4min	0
	F	-	-	5min	2min
Kidney, Nephropathy, Tubular Dilatation, Degeneration, vacuolar tubular	M	8min,2mil	8min,1mil	8 min,2mil	5min,1mil, 2mod
	F	2min	7min,1mil	5min, 1mil	3min, 3mil, 2mod
	M	0	0	0	2mil
	F	1min	1min	6min	2min,1mil
	M	0	0	0	1min
	F	0	0	0	1mil
Ureter Hyperplasia, transitional cells,	M	0	-	-	1/10
	F	0	-	-	0
	M	0	-	-	1/10
	F	0	-	-	0
Pituitary, adenoma Vacuolated cytoplasmic	M	0	-	1	0
	M	0	-	1/15min	2/15min
Lymph node, mesenteric Dilatation, sinuses, Histocytosis, sinuses Vacuolated macrophages, Lymphoid depletion	M	4/15min,4/15mil	-	-	7min
	F	4mil	-	-	3min,1mil
	M	4min	-	-	6min,1mil
	F	6min	-	-	1min
	M	0	-	-	2min,4mil
	F	0	-	-	1min, 2mil, 1mod
Lymph node, Submandibular Vacuolated macrophage,	M	1min	0	1min	8min, 3mil, 1mod
	F	0	0	3min, 1mil	12min,
Ovaries Increased vacuol. Interstitial cells	F	0	-	-	2 min
Spleen, Vacuolated macrophages	M	0	-	-	4min,1mil
	F	0	-	-	
Uterus, Vacuol. Cytoplasmic	F	0	-	-	2mil
Injection site inflamm., chronic active, Vacuol./necrosis, skeletal muscle Vacuol. Macroph. hypodermis,	M	1min	7min,1mil	2min,2mil, 9mod, 1sev	1min, 6mil, 5mod, 1sev
	F	1min	4min	3min, 5mil	1min, 8mil, 5mod
	M	0	1min,	1min, 4mil,1mod	2min,11mil
	F	0	0	2min	6min,7mil
	M	0	3min, 1mod	2min, 6mil, 7mod	2min, 7mil, 4mod
	F	0	6min	5min, 8mil	9mil, 5mod

min = minimal, mil = mild, mod = moderate, sev = severe

In recovery animals, nephropathy was seen in HD males (3/5 min, 1/5 mod) and females (2/5 mil). Fibrosis was still present at the injection site of males (1/5 min, 3/5 mod) and females (3/5 mil). Mild vacuolation macrophages of injection site was present in 4/5 males and females. Vacuolated macrophages were also noted in lymph notes of both HD males and females. The cytoplasmic vacuolation of the liver persisted in the 30 mg/kg/day male and female rats. One MD male had firm moveable mass (preputial gland, chronic active inflammation) in urogenital area during recovery.

Histopath findings in rats after 4 week recovery, WK 30:

Tissue	Sex	Dose, mg/kg/day			
		0 (4 M,F)	3 (4 M,15F)	10 (5 M,F)	30 (5 M,F)
Kidney, Nephropathy,	M	2min	4min	2min, 2mil	3min, 1 mod,
	F	0	7min,1mil	1min, 3mil	2min, 2mil,
	M	0	0	1min	1min
Tubular Dilatation,	F	0min	1min	0	0

min = minimal, mil = mild, mod = moderate

**Toxicokinetics:**

Tmax occurred at 8 hr after dosing for all dose groups. Although the Cmax values for females were higher than the Cmax values for males on Day 0, the variability among animals was high enough to preclude a definitive assessment of this relationship. Day 90 Cmax data, the relatively slight variations in concentration over the course of sampling suggest that the serum concentrations of B2036-PEG were near steady-state (table below).

On Day 178, serum concentrations of B2036-PEG typically remained relatively constant throughout the sampling period (See figure below), again suggesting that the serum concentrations of B2036-PEG were near steady-state. Although the Cmax values for MD and HD groups were higher on Day 178 than on Day 90, the increase was not as great as the increase between Day 0 and Day 90, again suggesting that the serum concentrations of B2036-PEG were near steady-state.

On all sampling days, the increase in Cmax with increasing dosage was proportional to the increase in dosage, however, the proportionality constant (ratio of Cmax to dose) increased during the course of the study.

Data calculated from plasma B2036-PEG measured by IRA		3 mg/kg/d		10 mg/kg/d		30 mg/kg/d	
		M	F	M	F	M	F
AUC <sub>0-8</sub> , µg.hr/ml	Day 0	12	18	36	58	116	160
	Day 90	277	455	945	1146	3820	2753
	Day 178	316	470	1671	1561	5142	3496
Cmax, µg/ml	Day 0	3.94	4.52	9.49	14.9	28.6	42.5
	Day 90	43.4	73.9	166	166	662	422
	Day 178	60.8	65.8	268	218	709	471

The AUC values were not provided by the sponsor. AUC values were calculated from mean plasma values by trapezoid method for comparative reasons only. Since the last blood sample in rats was taken at 8-hr post dose, a useful comparisons to human AUC (AUC<sub>0-inf</sub>) could not made. Plasma concentrations of B2036-PEG were inconsistent see tables below:

**Mean B2036-PEG Concentrations in Serum of Male Rats**

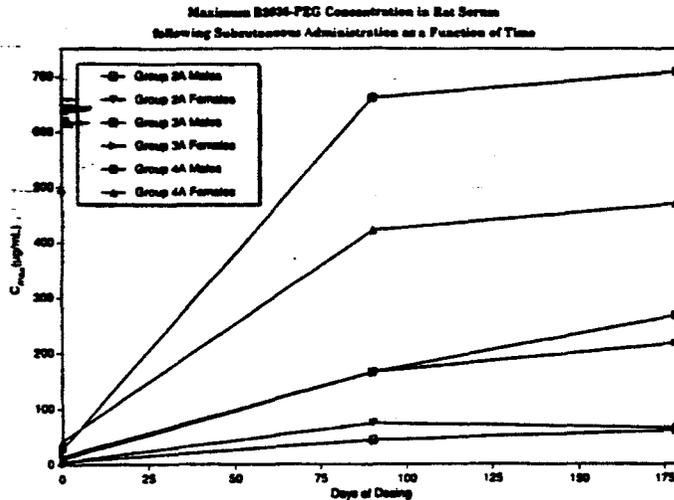
Group Summary			
Group	2A	3A	4A
Dose (mg/kg/day)	3	10	30
Mean (±SD) Serum Concentrations (µg/mL) - Day 0*			
0 hr	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
0.5 hr	0.150 (0.044)	0.504 (0.23)	1.74 (1.0)
1 hr	0.153 (0.028)	0.795 (0.23)	3.92 (3.2)
2 hr	0.334 (0.18)	1.34 (0.15)	3.97 (1.2)
4 hr	1.13 (0.17)	4.79 (1.8)	16.4 (5.4)
8 hr	3.94 (0.78)	9.49 (1.8)	28.6 (4.6)
Mean (±SD) Serum Concentrations (µg/mL) - Day 90*			
0 hr	39.1 (35)	136 (59)	506 (48)
0.5 hr	39.1 (21)	186 (72)	467 (272)
1 hr	43.4 (28)	118 (44)	483 (100)
2 hr	41.6 (32)	143 (77)	662 (24)
4 hr	40.1 (22)	96.5 (22)	457 (80)
8 hr	18.3 (4.4)	130 (30)	362 (104)
Mean (±SD) Serum Concentrations (µg/mL) - Day 178*			
0 hr	60.7 (28)	190 (32)	565 (116)
0.5 hr	42.9 (28)	232 (89)	491 (223)
1 hr	43.0 (28)	174 (26)	709 (306)
2 hr	60.8 (33)	203 (41)	617 (201)
4 hr	40.9 (26)	179 (26)	692 (257)
8 hr	16.7 (9.3)	268 (35)	611 (67)

\*Derived from Phoenix International report.  
N/A = not applicable. Tmax at 8 hr.

**Mean B2036-PEG Concentrations in Serum of Female Rats**

Group Summary			
Group	2A	3A	4A
Dose (mg/kg/day)	3	10	30
Mean (±SD) Serum Concentrations (µg/mL) - Day 0*			
0 hr	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
0.5 hr	0.199 (0.079)	0.811 (0.84)	1.51 (0.52)
1 hr	0.450 (0.088)	1.15 (0.60)	2.64 (1.4)
2 hr	0.610 (0.38)	1.67 (0.82)	4.96 (0.36)
4 hr	2.45 (0.68)	8.07 (1.7)	21.5 (5.2)
8 hr	4.52 (1.1)	14.9 (6.5)	42.5 (9.2)
Mean (±SD) Serum Concentrations (µg/mL) - Day 90*			
0 hr	58.1 (20)	121 (71)	212 (39)
0.5 hr	49.9 (7.9)	150 (108)	319 (26)
1 hr	73.9 (19)	166 (59)	422 (85)
2 hr	62.0 (29)	151 (81)	298 (86)
4 hr	59.2 (14)	154 (32)	393 (90)
8 hr	59.8 (3.8)	114 (49)	299 (49)
Mean (±SD) Serum Concentrations (µg/mL) - Day 178*			
0 hr	65.8 (24)	218 (66)	368 (56)
0.5 hr	50.9 (3.2)	204 (80)	362 (73)
1 hr	53.5 (20)	208 (54)	465 (95)
2 hr	62.8 (24)	182 (52)	372 (40)
4 hr	57.8 (23)	217 (51)	471 (81)
8 hr	60.3 (2.4)	182 (108)	447 (33)

\*Derived from Phoenix International report.  
N/A = not applicable. Positive slope.



Plasma concentrations appeared to reach steady state after repeated administration of B2036-PEG in rats. The human exposure ratio based on plasma concentration at steady state ( $C_{ss}$ , see table below)  $C_{ss}$  at NOEL dose of 3 mg/kg/day was similar to exposure ratio based on body surface area (1.5X).

Dose, mg/kg/d	Sex	$C_{ss}$ , µg/ml (day 90/178)	Multiple of human $C_{ss}$ with 20 mg/day (21-27 µg/ml)
3	M	40	1.5-2X NOEL 2-3X NOEL
	F	60	
10	M	170	6-8X 6-8X
	F	171	
30	M	553	20-26X 14-18X
	F	370	

#### Key Findings:

- HD males had significantly lower body weight at Week 13 (-9.5%) and Week 26 (15%). No significant change in food intake was noted.
- Platelets decreased (11%) in MD and HD males. White blood cells and lymphocytes decreased (> 20%) in HD males.
- Alkaline phosphatase levels decreased with all doses in males and in HD females.
- Liver and kidney weight in HD females was significantly increased. The increase in kidney weight appeared to correlate with nephropathy and tubular dilatation in HD female rats.
- There were significant dose-dependent increase in adrenal weight in males at all doses of B2036-PEG.
- There was a significant increase in proteinuria with 10 and 30 mg/kg dose. During recovery, proteinuria did not disappear suggesting renal damage was not readily reversible.
- Proteinuria and nephropathy in MD and HD females was confirmed by histological examination of renal tissue. Frequency of tubular dilatation (minimal) was 2/10 and 3/10 in MD and HD females, respectively.
- Thickening at the injection site increased dose-dependently. Four males in HD recovery also had thickened skin.
- All B2036-PEG treated rats exhibited inflammation, macrophage vacuolation and necrosis at the injection site. Since this was rarity in control, this was attributed to PEGylation. Other studies have reported similar findings with pegylated compounds or PEG alone.
- $C_{max}$  levels were higher in females than males. The increase in  $C_{max}$  was proportional to increase in dosage.

- NOAEL was 3 mg/kg/day (1.5 x human exposure based on body surface area). The human exposure based on C<sub>ss</sub> (21-27 µg/ml at 20 mg/day in humans) were 1.5 to 2 fold the C<sub>ss</sub> at NOAEL dose in male and female rats, respectively. The safety margins based on C<sub>ss</sub> confirms the exposure ratio based on body surface area.
- The higher dose of 10 and 30 mg/kg/day in rats produce exposures (C<sub>ss</sub>) 6-8X and 16-26 X C<sub>ss</sub> exposure with the maximum recommended human dose of 20 mg/kg.

#### Summary of individual study findings:

In this 6-month study, daily doses of 0, 3, 10 and 30 mg/kg B2036-PEG were administered to rats, SC. Only the highest dose in males significantly lowered body weight (Wk 13, -9.5%, Wk 26, -15%). No significant change in food intake was noted in any group. Both MD and HD males had lower WBC, lymphocytes and platelets. As reported in earlier studies in monkeys, alkaline phosphatase levels decreased in a dose-dependent manner. The 30 mg/kg/d dose significantly increased liver and kidney weight in females but not in males. There was significant dose-dependent increase in adrenal weight in males at all dose levels of B2036-PEG. The significance of the increase in adrenal weight is not clear. In the 26-week monkey study, an increase in vacuolation of zona fasciculata of adrenals in HD (3 mg/kg/wk) males and females monkeys were noted.

Both 10 and 30 mg/kg/d doses significantly increased the proteinuria in chronic rat study. During recovery, proteinuria did not disappear suggesting renal effects during treatment period were not readily reversible. Histological examination of the renal tissue found tubular dilatation and nephropathy in MD and HD females, respectively.

Other histological findings were specific to injection site. Similar to other toxicity studies, thickening of the skin at the injection site increased in a dose-dependent manner. All B2036-PEG treated rats exhibited inflammation, macrophage vacuolation and necrosis at the injection site. Since this was rarity in control, this reviewer attributed to PEGylation. Injection site sensitivity with pegylated compounds has been reported in literature. Toxicokinetic data found C<sub>max</sub> levels to be higher in females than males. The higher peak plasma level may account for increased nephropathy in female rats. The increase in C<sub>max</sub> was proportional to increase in dosage. The NOAEL was 3 mg/kg/day in females (1.5 times human exposure based on body surface area). Mean plasma levels at steady state (day 90 or 170) in rats with 3 mg/kg/d were 40 µg/ml in males and 60 µg/ml in females rats. Trough pegvisomant concentrations in acromegalic subjects treated with highest dose of 20 mg/day were 21-27 µg/ml therefore, the actual C<sub>ss</sub> data from rats and man confirm the safety margins as 1.5X for the rats NOAEL. The higher dose of 10 and 30 mg/kg/d in rats produce exposure (C<sub>ss</sub>) 6-8 and 14-26 times C<sub>ss</sub> exposures with the maximum recommended human dose of 20 mg/day.

#### Toxicology summary:

In the 28-Day rhesus monkey study, doses of 0, 0.1, 0.3, 1 or 3 mg/kg/SC were administered every other day (14 days total). Animals in the 1 and 3 mg/kg/d dose had lower body weights (-15 and -12%) and HD monkeys had slight (8-16%) decrease in RBC, Hgb, and PCV. Monkeys dosed with 1 and 3 mg/kg had decreased alkaline phosphatase. The NOAEL was 0.3 mg/kg, which produced exposure 1/3 human dose based upon body surface area. Importantly, there were no gross or histological findings except injection site swelling. **No treatment-related changes in urine parameters or in organ weight changes were observed.** Necropsy findings were limited to incidence of swelling at the site of injections. There were no other gross or histopathological findings in the 28-Day monkey study.

In a 6-month monkey study, B2036-PEG was administered once a week at doses of 0, 0.3, 1 and 3 mg/kg/wk, SC (see page 80). There was a very definite, treatment-related body weight loss compared to starting weights in both MD and HD animals of both sexes. This appeared to recover in males after 8 week drug-free period. The recovery was not clear in females. Although HD animals tended to eat less than controls, the change in food consumption was not apparently the

full cause of weight loss. At the HD, both sexes had decreases in hemoglobin, packed cell volume and RBC counts. There was also a trend for decreased platelets in the MD and HD. Males recovered from these changes, but one HD female still had low hemoglobin, PCV and RBC at the end of the 8 week recovery period. It is not clear if this was related to the weight changes. Monkeys treated with B2036-PEG doses  $\geq 1$  mg/kg had lower serum phosphorus and lower serum ALP. Blood urea was slightly increased in HD males and MD and HD females but recovered. Some of the histological findings included: vacuolation of zona fasciculata of adrenals in HD group, less prominent islets of Langerhans at HD all animals, increase in fatty infiltration in

**Histopathology Findings in Monkeys Dosed for 26 Weeks (SEN-109)**

Tissue/Finding Dose, mg/kg/week <sup>a</sup>	Males				Females			
	0	0.3	1	3	0	0.3	1	3
Colon/Cecum								
Submucosal fat	0	0	1	3	0	0	1	1
Kidney basophilic tubules	0	1	0	0	0	1	0	1
Interstitial inflammation	0	1	1	2	2	0	3	2
Thymus, fat infiltration	0	2	2	3	0	1	2	3
involution	0	0	2	2	0	0	1	0
Femur   trabecular bone	0	0	4	4	0	0	0	4
bone marrow	0	0	4	2	0	0	3	4
Sternum, thinning bone	0	0	4	4	0	0	0	2

a. Monkeys (n = 4/sex/dose) were administered 0, 0.3, 1, or 3 mg/kg once per week for 26 weeks. These doses are equivalent to 3.6, 12 and 36 mg/M<sup>2</sup>/week.

Maximum recommended human dose is 20 mg/day = 12 mg/M<sup>2</sup>/day x 7 = 84 mg/M<sup>2</sup>/week. Therefore, doses tested in monkeys are all fractional exposures (1/23, 1/7, and 1/3, respectively) of human therapeutic exposures. NOAEL was 0.3 mg/kg/week, approximately 1/23 human exposure with the maximum recommended human dose of 20 mg/day.

colon, cecum, thymus; an increase in glycogen vacuolation in the liver; reduction in bone of the body of the sternum and reduction in trabecular bone in the femur. This was accompanied by diminution of the marrow of the femur, particularly in the femoral head of MD and HD animals. The bone effects were still observed in the recovery animals.

Since B2036-PEG effectively binds to GH receptors in monkeys, as expected, IGF-1 was clearly lowered by 1 and 3 mg/kg dose at 1, 13 and 26 weeks. However, there was no effect on prolactin levels. Insulin levels tended to be reduced but were very variable and difficult to attribute to treatment.

Treated animals, but not controls, had a small incidence of injection site lesions consisting of induration in 2 animals each at 1 and 3 mg/kg/week. Swelling at injection sites was also observed in several animals of both sexes at these same doses. Overall NOAEL was 0.3 mg/kg on a weekly basis (~ 3.6 mg/m<sup>2</sup>/week). The doses studied are 1/23, 1/7 and 1/3 human therapeutic exposures based on body surface area.

In the 6-month study, daily doses of 0, 3, 10 and 30 mg/kg B2036-PEG were administered to rats, SC. Only the highest dose in males significantly lowered body weight (Wk 13, -9.5%, Wk 26, -15%). No significant change in food intake was noted in any group. Both MD and HD males had lower WBC, lymphocytes and platelet counts. Alkaline phosphatase levels decreased in a dose-dependent manner. The 30 mg/kg dose significantly increased liver and kidney weight in females but not in males. There was significant dose-dependent increase in adrenal weight of males starting with 3 mg/kg/d. The significance of the increase in adrenal weight is not clear. In the 26-week monkey study an increase in vacuolation of zona fasciculata of adrenals in HD (3 mg/kg/wk) males and females was noted.

Both 10 and 30 mg/kg/d doses significantly increased proteinuria in the chronic rat study. During recovery, proteinuria appear did not disappear suggesting significant renal damage was sustained during the recovery period. Histological examination of the renal tissue found tubular dilatation

(minimal) in 2/10 and 3/10 in MD and HD females, respectively. In the 6-month monkey study, only one male in HD group had minimal proteinuria at WK 26.

Other histological findings were specific to injection site. As it has been reported in other toxicity studies, the thickening at the injection site increased in a dose-dependent manner. All B2036-PEG treated rats exhibited inflammation and hypodermic macrophage vacuolation at the injection site. Since this was rarity in control, this reviewer attributed to PEGylation. Injection site sensitivity with pegylated compounds has been reported in literature. Toxicokinetic data found a Cmax levels to be higher in females than males. The higher peak plasma level may account for increased nephropathy in female rats. The increase in Cmax was proportional to increase in dosage. The NOAEL was 3 mg/kg/day in females (1.5 times human exposures with the maximum recommended human dose of 20 mg/day up on body surface area or C<sub>ss</sub> concentrations).

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Addendum

Histopathology Inventory for NDA # 21-106

Study No.	SEN-118
Species	26 WK Rat
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	X*
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	X
Harderian gland	X
Heart	X*
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X*
Knee Joint	X
Lacrimal gland	X
Larynx	
Liver	X*
Lungs	X*
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Medulla Oblongata	X
Mammary Gland	X
Nasal cavity	
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X*
Peripheral nerve	X
Pharynx	
Pituitary	X*
Prostate	X*
Rectum	X
Salivary gland	X*
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum	X
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X*
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X*
Vagina	X
Zymbal gland	

X, histopathology performed, \*,organ weight obtained

## GENETIC TOXICOLOGY:

**Study title:** Structural chromosomal aberration assay in human lymphocytes with B2036-PEG.

**Key findings:**

**Study no:** SEN-202

**Study type (if not reflected in title):** Chromosomal aberration

**Volume #, and page #:** 1.7, 1-38

**Conducting laboratory and location:**

**Date of study initiation:** Sep 23, 1998

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Lot # 372753A and assumed 100% purity

**Formulation/vehicle:** B2036-PEG was prepared with sterile water

### **Methods:**

**Strains/species/cell line:** human lymphocytes

**Dose selection criteria:** cytotoxicity (mitotic index)

**Basis of dose selection:** Cytotoxicity study showed that concentrations as high as 5000 µg/ml were not toxic. So three concentrations levels 500, 2500 and 5000 µg/ml were used in the main study.

**Range finding studies:** In the initial studies, doses 0.167, 0.5, 1.67, 5, 16.7, 50, 167, 500, 1670 and 5000 were tested for cytotoxicity.

**Test agent stability:** determined by the sponsor to be stable for the duration of the study

**Metabolic activation system:** Aroclor 1254-induced male SD rat liver homogenate was used in the study. The S9 mix contained 10 mM MgCl<sub>2</sub>, 10 mM CaCl<sub>2</sub>, 30 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 50 mM sodium phosphate buffer (pH 7.4) and 10% (v/v) liver homogenate. Due to technical error, excessive NADP (5.33 µM) was used in the preparation S9 mix but this should not have altered the study since insufficient NADP is more critical.

### **Controls:**

**Vehicle:** Sterile water

**Negative controls:** Sterile water

**Positive controls:** Mitomycin C (MMC, at 0.100 or 0.250 µg/ml) for tests without activation and cyclophosphamide (40 µg/ml) for S9 activation

### **Exposure conditions:**

**Incubation and sampling times:**

#### *Treatment-Harvest Schedules*

	Tr	W	C	H
<b>Schedule I -S9</b>	0	48	53	71 73
	Tr	W	C	H
<b>Schedule II +S9</b>	0	48	53	71 73
	Tr	W	C	H
<b>Schedule III +S9</b>	0	48	53	94 96
	Tr	W	C	H
<b>Schedule IV -S9</b>	0	48	71	73
	Tr		C	H
<b>Schedule V -S9</b>	0	48	94	96
	Tr		C	H

Numbers = Time (hrs)  
C = Colcemid addition

Tr = Treatment  
H = Harvest

W = Wash/re-feed

Doses used in definitive study: 500, 2500 and 5000 µg/ml

Study design: Since B2036-PEG was found to have no cytotoxicity, only three concentrations were tested with different incubation and harvesting time (see incubation schedule on previous page). Minimum of 3 concentrations without cytotoxicity are needed for ChromAb test. The lowest concentration 500 µg/ml was chosen because lower concentrations did not show an appreciable mitotic indices for evaluation. Tests were carried out with and without S9 mixture. In the initial main study, schedule I and II were used. In the follow up study, schedule III, IV and V were used.

**Analysis:**

No. of replicates: Each concentration was run in duplicate cultures.

Counting method: Approximately 1000 cells per culture were examined for mitotic index.

200 well-spread metaphases were scored for the presence of chromosomal aberration for each concentration of B2036-PEG.

**Criteria for positive results:**

- a) it induces a statistically significant, dose-dependent increase in the frequency of aberrations/cell or in the proportion of aberrant metaphases; or
- 2) it induces a reproducible, statistically significant increase in either endpoint for at least one test article concentration at the same treatment/sampling interval.

**Summary of individual study findings:**

Study validity: Study is considered valid if a) the number of aberrant metaphases for the negative (solvent) is less than 9 and B) both positive controls induce significant increase ( $p > 0.05$ ) in the frequency of aberration/cell and in the proportion of aberrant metaphases, as compared to the negative control. All these parameters were met in the study.

**Study outcome:**

- No cytotoxicity was observed in any of the treatment schedule in presence or absence of metabolic activation system. There were no dose-dependent decreases in mitotic index observed.
- The B2036-PEG did not have a deleterious effect upon pH or osmolality at concentrations up to 5000 µg/ml.
- Results of the two independent experiments indicated that B2036-PEG did not induce any statistically significant or dose-dependent increases in the proportion of aberrant metaphases, or in the frequency of aberrations/cell, at any concentration using any treatment schedule.
- All negative control values were within acceptable ranges, and the positive controls induced statistically significant increases in the proportion of aberrant metaphases and in the frequency of aberrations/cell ( $p < 0.01$ ). Polyploid indices for all cultures treated with B2036-PEG, in the presence and absence of S9, also approximated negative control values (see table in the next page for data summary).

**Conclusion:** B2036-PEG did not cause chromosomal aberration in human lymphocytes under the test conditions and according to the criteria of the test protocol.

Summary of Results

Treatment Group	Dose (µg/ml)	MI Depression <sup>a</sup> (%)	Aberrant Cells <sup>b</sup>		Aberrations/cell (x̄ ± SD)
			Number	%	
<b>Schedule I (-S9; 5-hr treatment; 73-hr harvest)</b>					
SWFI <sup>c</sup>	10.0	NA <sup>d</sup>	1	0.5	0.005 ± 0.071
B2036-PEG	500	9.5	2	1.0	0.015 ± 0.158
B2036-PEG	2500	5.6	0	0.0	0.000 ± 0.000
B2036-PEG	5000	44.7	0	0.0	0.000 ± 0.000
MMC	0.250	74.9	42	42.0**	0.690 ± 1.116**
<b>Schedule II (+S9; 5-hr treatment; 73-hr harvest)</b>					
SWFI	10.0	NA	0	0.0	0.000 ± 0.000
B2036-PEG	500	31.4	1	0.5	0.005 ± 0.071
B2036-PEG	2500	19.6	1	0.5	0.005 ± 0.071
B2036-PEG	5000	- <sup>e</sup>	1	0.5	0.005 ± 0.071
CP	40.0	96.1	26	46.4**	0.857 ± 1.299**
<b>Schedule III (+S9; 5-hr treatment; 96-hr harvest)</b>					
SWFI <sup>f</sup>	10.0	NA <sup>d</sup>	2	1.0	0.010 ± 0.100
B2036-PEG	500	- <sup>e</sup>	2	1.0	0.010 ± 0.100
B2036-PEG	2500	-	1	0.5	0.005 ± 0.071
B2036-PEG	5000	11.4	0	0.0	0.000 ± 0.000
SWFI <sup>f</sup>	10.0	NA	3	1.5	0.015 ± 0.122
CP <sup>f</sup>	40.0	76.4	37	50.7**	0.781 ± 0.946**
<b>Schedule IV (-S9; 25-hr treatment; 73-hr harvest)</b>					
SWFI <sup>f</sup>	10.0	NA <sup>d</sup>	1	0.5	0.005 ± 0.071
B2036-PEG	500	- <sup>e</sup>	5	2.5	0.040 ± 0.262
B2036-PEG	2500	12.8	0	0.0	0.000 ± 0.000
B2036-PEG	5000	55.9	4	2.0	0.020 ± 0.140
MMC	0.100	-	28	28.0**	0.380 ± 0.678**
<b>Schedule V (-S9; 48-hr treatment; 96-hr harvest)</b>					
SWFI	10.0	NA	1	0.5	0.005 ± 0.071
B2036-PEG	500	-	0	0.0	0.000 ± 0.000
B2036-PEG	2500 <sup>f</sup>	12.4	2	1.0	0.015 ± 0.158
B2036-PEG	5000 <sup>f</sup>	53.5	1	0.5	0.005 ± 0.071

<sup>a</sup>As described in the text, 1000 cells were scored per concentration. Results represent the average of duplicate cultures. %MI depression is based on comparison to the SWFI negative control.

<sup>b</sup>As described in the text, 200 metaphase cells were scored per dose group (100 per replicate culture), except for the MMC positive control (100 cells/single culture).

<sup>c</sup>Sterile water for injection, µl/ml.

<sup>d</sup>Not applicable.

<sup>e</sup>No depression relative to SWFI negative control.

<sup>f</sup>A single metaphase with shattered chromosomes was recorded separately but not included in the total number of metaphases used for statistical analysis of the data.

\*\*Statistically significant increase (p ≤ 0.01) as determined by Chi-square (% aberrant cells) or Dunnett's analysis (aberrations/cell).

**Genetic toxicology Summary:** Sponsor had carried out only two genotoxicity test: Ames and Chromosomal aberration test. The Ames test was reviewed by Ron Steigerwalt. Under the test conditions, B2036-PEG did not cause reverse mutation of Salmonella or E.coli (negative Ames test). The chromosomal aberration test was also negative.

**Labeling recommendations:**

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## CARCINOGENICITY:

Standard 2-year rodent bioassays have not been performed. The sponsor conducted several *in vitro* and *in vivo* studies to evaluate the effects of pegvisomant on tumor cell lines responsive to growth hormone.

Study Title: The effect of B2036-PEG on human meningioma growth *in vitro*.

Study no: SEN-205

Study type: Alternative test to assess carcinogenic potential of B2036-PEG

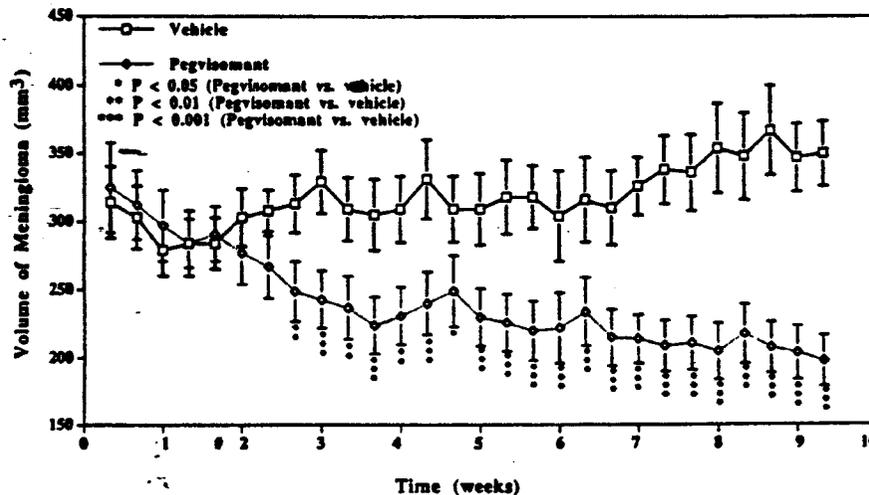
Conducting laboratory and location:

GLP compliance: No

Objective: To assess the ability of B2036-PEG to reduce serum-induced DNA synthesis in primary meningioma cultures was assessed *in vitro*. In addition, the ability of B2036-PEG to inhibit meningioma tumor growth in xenografted athymic (nude) mice was determined.

Methods: To evaluate the efficacy of GH receptor blockage in an *in vivo* system, primary cultures from human meningioma tumors were xenografted into athymic mice. A preliminary experiment using 8 human meningioma specimens was performed followed by a definitive study using 15 meningiomas. In each experiment, approximately 1.5 million cells from each of the tumors were implanted into the flanks of 2 female mice/tumor. One animal from each pair was treated with B2036-PEG while the other received vehicle alone. Treatment consisted of daily subcutaneous injections of 45 mg/kg B2036-PEG or an equivalent volume (0.2 mL) of vehicle for 8 weeks. During the treatment period, tumors were measured for volume three times each week. At the end of the study, tumors were excised and weighed. In addition, serum IGF-1 and IGFBP-3 concentrations were measured as well as IGF-1 and IGF-1I concentrations in the tumors.

Results: Blockade of the GH receptor with B2036 reduced serum-induced DNA synthesis by 8 to 33% with a mean reduction of 20% in primary meningioma cultures. Tumors that expressed the GH receptors i.e. GHRwt and GHRd3 isoforms or a combination of the two, were all responsive to treatment with B2036. In the pilot study, during Week 2 of treatment with B2036-PEG, mean tumor volume was reduced with statistical significance obtained consistently during Weeks 3 to 10. In the main study, the mean tumor volume was significantly reduced by B2036-PEG. Figure below shows the effect of B2036-PEG on the growth of tumor xenografted into nude mice (main study, 15 tumors/data point).



Tumor weight in the B2036-PEG-treated group was  $0.067 \pm 0.01$  g compared to  $0.092 \pm 0.01$  g in the vehicle control group ( $p < 0.2$ ). Mean serum IGF-1 levels are shown in table below:

IGF-I, IGF-II and IGFBP measurements in treatment groups		
	Vehicle	Pegvisomant
<b>Serum Measurements</b>		
IGF-I ( $\mu\text{g/L}$ )	$319 \pm 13.0$	$257 \pm 9.7^*$
IGFBP-1 (pixel intensity)	$28.6 \pm 3.00$	$47.5 \pm 7.61^{**}$
IGFBP-2 (pixel intensity)	$177 \pm 30.6$	$232 \pm 37.4$
IGFBP-3 (pixel intensity)	$469 \pm 24.6$	$436 \pm 20.3^*$
IGFBP-4 (pixel intensity)	$73.3 \pm 14.1$	$135 \pm 20.7^{**}$
<b>Tumor Measurements</b>		
IGF-1 (ng/g tumor)	Not Detectable	Not Detectable
IGF-II (ng/g tumor)	$8.8 \pm 4.3$	$13.8 \pm 5.4$ (N.S.)

\*  $P < 0.03$   
 \*\*  $P < 0.01$

**Summary:** Activation of the GH/IGF-1 axis significantly increases the growth rate of human meningioma. Blockage of the GH receptor on tumor cells with B2036 inhibited tumor growth. GH receptor appears to be expressed ubiquitously in human meningioma tumors. Down-regulation of the GH/IGF-1 axis with B2036-PEG significantly decreases the growth rate of human meningioma in nude mice. Accompanying the decreased growth rate is a modest decrease (20%) in serum IGF-1 concentration.

**Study Title:** The effect of B2036-PEG on breast cancer cell lines

Study No: SEN-206

Study type: Alternative test to assess carcinogenic potential of B2036-PEG

Conducting laboratory and location:

**GLP compliance:** No

**Objective:** To assess the effect of IGF-I on the growth rates of a number of breast cancer cell lines and the antagonistic effect of B2036-PEG administration on tumor growth in nude mice into which tumor cells were implanted.

**Methods:** Athymic (nude) mice were implanted in the flank with various tumor cell lines, and mice were then treated with B2036-PEG. Three estrogen receptor positive (E+) cell lines (MCF-7, T-47-D, ZR-75-1) and one estrogen receptor negative (E-) cell line (MDA-MB-231) were used in the study. For tumor lines MDA-MB-231 (5 million cells), T-47-D (12 million cells), MCF-7 (28 million cells) and ZR-75-1 (12 million cells) were injected into each nude mouse. They were either treated with B2036-PEG (202.5 mg/kg/week in 3 divided dose each week) or vehicle (buffer for reconstitution). Tumor volume ( $\text{mm}^3$ ) was measured 3 times each week using precision calipers. Drug treatment duration was between 8 to 14 weeks depending on the cell lines.

**Results:**

- GH receptor mRNA was present in 4 of 5 cell lines tested. However, IGF-1 mRNA was not expressed by any of the cell lines, demonstrating that no autocrine production of IGF-1. IGF-1 receptor mRNA was expressed in all cell lines.
- Based on the in vivo studies, B2036-PEG administration leads to a reduction in breast cancer cell line growth rate, especially in cell lines T-47-D and MCF-7.

- The amount of the reduction of the circulating IGF-1 concentrations observed in the B2036-PEG animals was approximately 20% for the T-47D, MCF-7 and MDA-MB-231 cells.

The mean tumor volume (mm<sup>3</sup>) and IGF-1 levels measured at the end of week 8 are shown in table below. The tumor size was measured 3 times a week by precision caliper.

#### Effects of B2036-PEG on Breast Cancer Models<sup>a</sup>

<u>Tumor Type</u>	<u>B2036-PEG</u>	<u>Vehicle</u>	<u>p value</u>
MDA-MB-231	628.2 ± 189.3 <sup>b</sup>	913.6 ± 390.5	.2699
ZR-75-1	204.8 ± 31.9	293.0 ± 50.2	.3126
T-47-D	155.3 ± 9.6	277.0 ± 25.0	.0010
MCF-7	165.0 ± 12.0	293.1 ± 25.8	.0048
<u>Serum IGF-I Concentrations (µg/L)</u>			
MDA-MB-231	258.2 ± 18.6	322 ± 11.5	.0211
ZR-75-1	277.4 ± 11.4	277.4 ± 14.5	N/A
T-47-D	280.0 ± 17.2	336.7 ± 10.1	.0430
MCF-7	244.4 ± 23.9	321.3 ± 11.5	.0331
<u>Tumor IGF-II Concentrations (ng/g tumor)</u>			
MDA-MB-231	3.1 ± 0.4	10.3 ± 2.6	.0366

Summary: Breast cell lines used in this study expressed the receptors necessary to respond to either GH and/or IGF-1. None of the cells had autocrine function to produce IGF-1. Based on this in vivo study, the B2036-PEG reduced growth rate of T-47-D and MCF-7 breast cancer cell lines.

Study Title: Therapy of experimental liver metastases produced by murine CT-26 colon carcinoma with irinotecan (topoisomerase I inhibitor) with or without the growth hormone receptor antagonist, pegvisomant (B2036-PEG).

STUDY NO: SEN-207

Study type: Alternative test to assess carcinogenic potential of B2036-PEG

Conducting laboratory and location:

GLP compliance: No

Objective: To assess the effect of B2036-PEG on colon cancer tumors transplanted into mice.

Methods: BALB/c mice were injected into the spleen with  $1 \times 10^4$  viable CT-26 cells and then treated with B2036-PEG (30 mg/kg, sc) once daily for 23 days. On post-inoculation days 7, 14 and 21, groups of mice received injections of irinotecan, a topoisomerase I inhibitor (CPT-11, 100 mg/kg). Mice were sacrificed on Day 24, the splenic tumors were removed, and the volumes measured. In addition, liver weights were obtained and the hepatic metastases were counted. Prior to the study, CT-26 cells were measured for growth hormone (GH), IGF-1 and IGF-1I receptor mRNA. Approximately 40% of the tumor specimens expressed growth hormone receptor mRNA.

Results:

- Tumors in the spleen were noted in all transplanted mice on Day 24.
- The splenic tumor volume was reduced with B2036-PEG.

- The incidence of hepatic metastases was 8/10 for vehicle-treated animals and 7/10 for B2036-PEG-treated animals.
- The hepatic metastasis incidence fell to 4/10 for the mice receiving B2036-PEG and CPT-11.
- The mean number of hepatic metastases was significantly reduced by B2036-PEG alone or in combination with CPT-11.
- B2036-PEG combined with CPT-11 significantly reduced mean liver weight.

Treatment	Splenic Tumor		Liver Metastasis		Liver Weight (grams)
	Incidence	Mean Tumor Volume (mm <sup>3</sup> )	Incidence	Median#/Mean# (range)	
Vehicle	10/10	839.8 ± 173.8	8/10	14/43	2.27 ± 0.6
CPT-11	10/10	607.1 ± 97.9	8/10	55/48.2	2.08 ± 0.2
B2036-PEG	9/10	660.9 ± 115.6	7/10	12/29.1 <sup>ab</sup>	2.03 ± 0.3
B2036-PEG + CPT-11	9/10	307.6 ± 120.9 <sup>a</sup>	4/10	0/11.5 <sup>abc</sup>	1.39 ± 0.1 <sup>b</sup>

<sup>a</sup> p < 0.05 as compared to vehicle

<sup>b</sup> p < 0.05 as compared to CPT-11

<sup>c</sup> p < 0.05 as compared to B2036-PEG

Summary: B2036-PEG administration appeared to lower number of liver metastases when compared to vehicle-treated or CPT-11-treated animals. When CPT-11 and B2036-PEG were co-administered, treatment effect was significantly better than either treatment alone.

#### Carcinogenicity toxicology summary:

Sponsor has not completed any type of chronic carcinogenicity study. The agency had agreed to the submission of carcinogenicity study during phase IV development/post marketing period. In lieu of carcinogenicity studies, sponsor has submitted several in vitro and in vivo (non-GLP) mutagenicity/carcinogenicity studies with B2036-PEG. In these studies, B2036-PEG, was given to mice bearing human meningioma tumors, 4 lines of human breast tumors and murine colon tumors.

In mice transplanted with human meningioma tumors, B2036-PEG significantly decreases the growth rate of human meningioma in nude mice. Accompanying the decreased growth rate is a modest decrease (20%) in serum IGF-1 concentration. Blockage of the GH receptor on tumor cells with B2036 inhibited tumor growth. In addition, activation of the GH/IGF-1 axis significantly increases the growth rate of human meningioma. In breast cancer cell line study, B2036-PEG reduced growth rate of T-47-D and MCF-7 breast cancer cell lines. Finally, B2036-PEG administration to rats injected with colon cancer cells, the number of liver metastases was lower when compared to vehicle-treated or CPT-11-treated animals. When CPT-11 (topoisomerase I inhibitor) and B2036-PEG were co-administered, treatment effect was significantly better than either treatment alone. The mechanism of B2036-PEG anti-tumor activity is suggested to be through the GH/IGF-1 axis. GH/IGF-1 axis is important in human tumor growth. These studies appear to suggest B2036-PEG could exert anti-tumor effects in patients. It is likely that B2036-PEG may not initiate or promote tumor growth but until actual carcinogenicity studies are done, it is only a speculation.

**Recommendation for future analysis:**

The sponsor should submit a protocol for a 2-year rodent carcinogenicity study for review by the Executive Carcinogenicity Assessment Committee prior to initiation of the study.

**Labeling Recommendations:**

Standard 2-year rodent bioassays have not been performed with pegvisomant.

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## REPRODUCTIVE TOXICOLOGY:

**Study Title:** A dose range-finding study of the effects of B2036-PEG administered subcutaneously on embryo/fetal development in rabbits (SEN-116).

B2036-PEG was administered to New Zealand white rabbits (6/group) by SC injection at dosages of 0 (vehicle) 0.3, 1, 3 or 10 mg/kg/day from gestation days (GD) 7 through 20 in a dose range-finding study. Blood samples were collected from all rabbits two hours following dosing on GD 14 and 20 and prior to sacrifice on GD 29. Samples were processed to serum and analyzed for GH and IGF-1 concentrations. The uteri and ovaries were examined and the number of viable and dead fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Fetuses were weighed and external malformation and developmental variations were examined.

Mean serum IGF-1 concentrations in the 10 mg/kg/day group were reduced relative to the control group on GD 14 and 20 while the concentrations in the 0.3, 1 and 3 mg/kg/day groups were similar to the control group values. On GD 29, IGF-1 concentrations in the 1, 3 and 10 mg/kg/day groups were increased in a non-dose-related manner relative to the control group.

Treatment groups	IGF-1, ng/ml		
	GD14	GD20	GD29
Control, Vehicle	134	105.2	63.8
0.3 mg/kg	169.2	119.8	63.5
1 mg/kg	213.2	166.2	102.7
3 mg/kg	104.7	106.5	83.7
10 mg/kg	82.7	54.2	97.3

Treatment groups	GH, ng/ml		
	GD14	GD20	GD29
Control, Vehicle	56.8	55.8	70.7
0.3 mg/kg	20.2	54.7	98.2
1 mg/kg	34.0	55.7	51.3
3 mg/kg	118.5	95.6	66.1
10 mg/kg	198.7	464.0	99.2

The IGF-1 concentration in the 0.3 mg/kg/day group on GD 29 was similar to that of the control group. Serum GH concentrations were increased at 3 and 10 mg/kg/day when compared to the control group on GD 14 and 20. On GD 29, the GH concentrations in these groups were similar to the control group while the GH concentrations at 0.3 and 1 mg/kg/day were similar to control values at all intervals tested in the study. **These data show that B2036-PEG administration at dosages of 3 and 10 mg/kg/day led to increased circulating GH concentrations and administration at 10 mg/kg/day led to decreased IGF-1 concentrations.**

Intrauterine growth and survival were not affected by any dose level. No external malformation or external developmental variations were noted in fetuses. This study, in conjunction with the findings observed in normal rabbits, demonstrate that the rabbit is a suitable species in which to perform reproductive development studies.

There were no changes in post-implantation loss. The slight decrease ( $p < 0.05$ ) in mean fetal weight in LD but not MD and HD was not considered drug related (see Table below).

PROJECT NO. 1  
 SPONSOR: PENDING  
 SPONSOR NO.: 1202-116

**R-7 STUDY OF B2036-PEG ON EMBRYO/PETAL DEVELOPMENT IN RABBITS  
 SUMMARY OF MEAN FETAL DATA AT THE SCHEDULED NECROPSY**

GROUP	VIBL FETURES		DEAD FETURES		DESCRIPTIONS		POST IMPLANTATION		CORPORA		PRE IMPLANTATION		FETAL WEIGHTS		NO. OF GRAVID FEMALE
	NO.	%	NO.	%	EARLY	LATE	LOSSES	SITES	LUTEA	LOSSES	IN GRAVE	MEANS	S.D.		
1 TOTAL	39	0	1	0	1	0	1	40	38	18	NA	NA	NA	4	
MEAN	6.3	0.0	0.2	0.0	0.2	0.0	0.2	6.7	9.7	3.0	49.9	49.9	4.92		
S.D.	1.87	0.00	0.41	0.00	0.41	0.00	0.41	1.63	2.25	2.30	2.30	2.30	4.92		
2 TOTAL	37	0	4	0	4	0	4	41	34	15	NA	NA	NA	4	
MEAN	6.2	0.0	0.7	0.0	0.7	0.0	0.7	6.8	9.3	2.5	42.9*	42.9*	4.75		
S.D.	1.94	0.00	0.82	0.00	0.82	0.00	0.82	2.14	2.42	1.38	4.75	4.75	4.75		
3 TOTAL	14	0	2	0	2	0	2	16	30	14	NA	NA	NA	4	
MEAN	3.5	0.0	0.5	0.0	0.5	0.0	0.5	4.8	7.5	3.5	30.9	30.9	2.88		
S.D.	2.00	0.00	1.00	0.00	1.00	0.00	1.00	2.31	2.38	2.38	2.88	2.88	2.88		
4 TOTAL	24	0	3	0	3	0	3	27	45	38	NA	NA	NA	4	
MEAN	6.0	0.0	0.8	0.0	0.8	0.0	0.8	6.8	14.3*	9.3	31.1	31.1	2.82		
S.D.	2.54	0.00	0.96	0.00	0.96	0.00	0.96	3.38	3.54	8.64	8.64	8.64	2.82		
5 TOTAL	21	0	3	0	3	0	3	24	44	20	NA	NA	NA	4	
MEAN	5.3	0.0	0.8	0.0	0.8	0.0	0.8	6.8	11.8	5.0	32.6	32.6	4.11		
S.D.	3.30	0.00	0.96	0.00	0.96	0.00	0.96	2.38	4.24	4.24	4.11	4.11	4.11		

\* = Significantly different from the control group at 0.05  
 NA = NOT APPLICABLE  
 MEAN NUMBER OF VIABLE FETURES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA,  
 FETAL WEIGHTS COMPARED USING GARNETT'S TEST  
 1- 0 MG/KG/DAY 2- 0.3 MG/KG/DAY 3- 1 MG/KG/DAY 4- 3 MG/KG/DAY 5- 10 MG/KG/DAY

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This study was initiated on Nov 5, 1998 and completed on March 3, 1999. The other two main studies were initiated before the dose-range finding study. It is unlikely that results from this study helped determine doses for the main studies.

Study title: A study of the effects of B2036-PEG administered subcutaneously on early embryonic development to implantation in rabbits (SEN-120)

Key study findings: No maternal toxicity at any dose level. Postimplantation loss at 10mg/kg/day.

Study no.: SEN-120  
 Volume #, and page #: 1.7, 1-143  
 Conducting laboratory and location:

Date of study initiation: November 2, 1998  
 GLP compliance: Yes  
 QA reports: yes ( X ) no ( )  
 Drug, lot #, radiolabel, and % purity: Lot # I1257018B (10 mg/ml) not radiolabeled and purity not known but assumed 100%.  
 Formulation/vehicle: B2036-PEG was in sterile solution of mannitol (18 g/l), glycine(0.68 g/l) and sodium phosphate monobasic (5 mM)

Methods:  
 Species/strain: New Zealand white rabbits  
 Doses employed: 0, 1, 3, 10 mg/kg/day (1, 3, 10 X human exposure based on mg/m<sup>2</sup>)  
 Route of administration: SC  
 Study design: Four groups of female rabbits (5 months old) were artificially inseminated. They received a dose volume of 1 ml/kg of vehicle or 3 dosage levels of the drug by SC route from Day 0 to Day 7 gestation. On day 21 of gestation, a laparotomy was performed. Animals were observed twice daily. Food intake was recorded daily. Body weights were measured on days 0, 4, 8, 12, 16 and 21.  
 Number/sex/group: 20 female rabbits/dose



Offspring:

- The incidence of early (32.2%) and total resorption (33%) increased in HD group.
- There were no differences in pre-implantation loss (%) among groups, however, they were higher than historical data from — research labs. This could be attributed to handling and control vehicle (18 g/L mannitol, 0.68 g/L glycine and monobasic sodium phosphate).
- The number of viable fetuses in the HD group were less than historical data.

PROJECT NO. \_\_\_\_\_ A STUDY OF 12354-PER ADMINISTERED BUNICAMIDE<sup>®</sup> TO RABBITS  
 SPONSOR: STELLER DRUG SUMMARY OF MEAN FETAL DATA AT SCHEDULED NECROPSY (X PER LITTER)  
 SPONSOR NO.: 12354-123

GROUP NUMBER:	1	2	3	4
<b>CORPORA LUTEA</b>				
MEAN	12.5	12.7	13.7	11.1
S.D.	4.38	3.92	7.79	3.43
N	14	12	17	16
<b>IMPLANTATION SITES</b>				
MEAN	5.8	5.9	5.4	5.1
S.D.	3.31	3.75	2.87	1.93
N	14	12	17	16
<b>VIABLE FETUSES (X)</b>				
MEAN	78.7	82.7	91.9	67.8
S.D.	34.93	28.25	14.48	34.84
N	14	12	17	16
<b>DEAD FETUSES (X)</b>				
MEAN	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00
N	14	12	17	16
<b>EARLY RESORPTIONS (X)</b>				
MEAN	21.3	15.2	7.3	32.3
S.D.	34.93	28.25	14.43	37.38
N	14	12	17	16
<b>LATE RESORPTIONS (X)</b>				
MEAN	0.0	2.1	0.0	0.7
S.D.	0.00	7.22	3.67	2.78
N	14	12	17	16
<b>TOTAL RESORPTIONS (X)</b>				
MEAN	21.3	17.3	8.1	33.0
S.D.	34.93	28.25	14.48	34.84
N	14	12	17	16
<b>PRE-IMPLANTATION LOSS (X)</b>				
MEAN	55.6	53.3	53.2	49.4
S.D.	30.57	25.16	29.54	21.34
N	14	12	17	16
<b>POST-IMPLANTATION LOSS (X)</b>				
MEAN	21.3	17.3	8.1	33.0
S.D.	34.93	28.25	14.48	34.84
N	14	12	17	16

1- 0 MG/EE/DAY 2- 1 MG/EE/DAY 3- 3 MG/EE/DAY 4- 10 MG/EE/DAY

PROPORTIONAL (X) DATA COMPARED USING THE KRUSKAL-WALLIS TEST  
 CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST  
 None significantly different from control group

Key Study Findings:

- No treatment related clinical findings (signs, body wt.) were noted in dams at any dose, suggesting that doses used were not high enough. The highest dose was only 10 times human dose.
- Five, eight, three and four females in the control, 1, 3 and 10 mg/kg/day groups, respectively were not pregnant. ICH guidelines recommend between 16 to 20 litters for evaluation.
- The doses used in the study were not high enough to produce maternal toxicity.
- Although the pituitary weights (g) were similar among groups (control, 0.044±0.01; LD, 0.425±0.009; MD 0.0410±0.014; HD 0.0367±0.006) there was a decreasing trend with increasing dose.
- There were 3, 3, 5 and 8 oviduct cysts in control, LD, MD and HD, respectively.
- The incidence of early (32.2%) and total resorption (33%) increased in HD group.
- Increase in postimplantation loss in HD group (10X human exposure based on body surface area).
- NOAEL was 3 mg/kg (3 times human exposure based upon body surface area).

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**Study title:** A study of the effects of B2036-PEG administered subcutaneously on early embryo/fetal development in rabbits (SEN-119)

**Key study findings:** No maternal or embryofetal toxicity or developmental variation at any dose level. The doses evaluated in the study were not high enough since maternal toxicity was not observed.

**Study no.:** SEN-119

**Volume #, and page #:** 1.7, 1-347

**Conducting laboratory and location:**

**Date of study initiation:** Aug 6, 1998

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Lot # 11257018B (10 mg/ml) not radiolabeled and purity not known but assumed 100%.

**Formulation/vehicle:** B2036-PEG was in sterile solution of mannitol (18 g/l), glycine (0.68 g/l), and sodium phosphate monobasic (5 mM), pH 7.4

**Methods:**

**Species/strain:** New Zealand white rabbits

**Doses employed:** 0, 1, 3, 10 mg/kg/day

**Route of administration:** SC

**Study design:** Four groups of female rabbits (5 months old) were artificially inseminated. They received a dose volume of 1 ml/kg of vehicle or 3 dosage levels of the drug by SC route from Day 7 to Day 20 gestation. On day 21 of gestation, a laparotomy was performed. Animals were observed twice daily. Food intake was recorded daily. Body weights were measured on days 0, 7-21 (daily), 24 and 29.

**Number/sex/group:** 22 female rabbits/dose

**Parameters and endpoints evaluated:** Evaluation included the uteri, ovaries, the early and late resorptions, total implantations and corpora lutea. Selected maternal tissues were collected for possible histological examination. The brain, ovaries and pituitary gland were weighted. Fetuses were weighed, sexed and examined for external, soft tissue and skeletal muscle malformation and developmental variation.

**Toxicokinetics:** Not available

**Results:**

**Mortality:** All females survived until the end of the study, gestation Day 21.

**Clinical signs:** There were no major treatment related clinical signs at any dose level. Clinical signs of hair loss, clear nasal discharge, injection site inflammation, edema, hemorrhage were seen in treated groups

STUDY OF B2036-PEG ON EMBRYO/FETAL DEVELOPMENT IN RABBITS  
GENERAL OBSERVATIONS: TOTAL INCIDENCE / NO. OF ANIMALS

TABLE RANGE: GROUP	10-00-98 TO 11-00-98			
	1	2	3	4
GENERAL OBS	488/22	488/22	488/22	488/22
-ALL FURS WERE SHED AFTER BIRTH	13/3	14/4	0/0	14/4
-SCAPULAR AREA	0/0	0/2	0/0	10/3
-SCAPULAR AREA, LEFT	4/1	0/2	11/3	4/3
-SCAPULAR AREA, RIGHT	0/0	13/4	0/4	0/2
-LUMBAR AREA	0/0	0/5	0/4	13/3
-LUMBAR AREA, LEFT	0/0	0/5	0/4	10/4
-LUMBAR AREA, RIGHT	0/0	3/2	0/4	0/3
-NO SPINA	0/0	0/0	7/4	2/2
-SCAPULAR AREA, LEFT	0/0	12/5	3/2	0/3
-SCAPULAR AREA, RIGHT	0/0	2/1	0/0	4/3
-LUMBAR AREA, LEFT	0/0	0/0	0/0	3/2
-LUMBAR AREA, RIGHT	0/0	0/0	4/2	2/1
-SCAPULAR AREA, LEFT	0/0	1/1	0/0	0/0
-SCAPULAR AREA, RIGHT	0/0	1/1	0/0	0/0
-LUMBAR AREA, LEFT	0/0	0/0	0/0	2/1

1- 0 mg/kg/day 2- 1 mg/kg/day 3- 3 mg/kg/day 4- 10 mg/kg/day

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Body weight: No change in body weight of treated vs. control.  
 Food consumption: No difference in food consumption

**For embryofetal development studies:**

In-life observations:

Terminal and necropsy evaluations:

- No drug-related findings were observed at any dose level.
- Intrauterine growth and survival were not affected by B2036-PEG.

Dams:

- The increase in postimplantation loss in HD group was not statistically significant in part because all groups were high relative to historical data from \_\_\_\_\_ Sponsor attributed increase in post-implantation loss to low number of implantation sites in several animals rather than test compound. Since the loss was nearly two fold higher than control and previous study found a similar increase in postimplantation loss, the reviewer believes it is drug related.
- The number of females with litter losses (3, 2, 3 and 3 in control, 1, 3 and 10 mg/kg group) were high in all groups, but within historical data.

PROJECT NO.: \_\_\_\_\_ STUDY OF B2036-PEG ON EMBRYO/FETAL DEVELOPMENT IN RABBITS  
 SPONSOR: SENSIL/DRLS SUMMARY OF MEAN FETAL DATA AT THE SCHEDULED NECROPSY  
 SPONSOR NO.: 15EN-117

GROUP	SEX		VIABLE FETUSES	DEAD FETUSES	RESORPTIONS		POST IMPLANTATION LOSS		CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES	
	M	F			EARLY	LATE	LOSS	SITES					
1	TOTAL	48	41	89	0	9	1	10	99	176	75	NA	19
	MEAN	2.5	2.2	4.7	0.0	0.5	0.1	0.5	9.2	9.2	3.9	51.2	
	S.D.	2.17	2.01	3.27	0.00	0.77	0.23	0.77	2.92	2.75	2.68	6.82	
2	TOTAL	64	66	128	0	11	1	12	140	287	67	NA	18
	MEAN	3.6	3.6	7.1	0.0	0.6	0.1	0.7	7.8	11.5	3.7	45.7	
	S.D.	1.95	1.92	3.23	0.00	1.49	0.24	1.68	2.37	3.24	2.52	6.35	
3	TOTAL	62	40	102	0	9	3	12	114	202	88	NA	18
	MEAN	3.4	2.2	5.7	0.0	0.5	0.2	0.7	6.3	11.2	4.9	48.5	
	S.D.	2.61	1.80	3.61	0.00	0.62	0.38	0.84	3.41	3.44	4.01	6.98	
4	TOTAL	42	52	94	0	23	0	23	117	204	87	NA	19
	MEAN	2.2	2.7	4.9	0.0	1.2	0.0	1.2	6.2	18.7	4.6	47.4	
	S.D.	1.99	2.23	3.12	0.00	1.47	0.00	1.47	2.54	4.09	3.58	5.56	

None significantly different from control group

NA = NOT APPLICABLE

MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING GUNNETT'S TEST

1- 0 MG/KG/DAY 2- 1 MG/KG/DAY 3- 3 MG/KG/DAY 4- 10 MG/KG/DAY

Offspring:

- External malformations were observed in 2(2), 3(1), 0(0) and 1(1) fetuses (litters) in control, 1, 3 and 10 mg/kg groups, respectively.
- No treatment-related visceral development variations were observed at any dose group.
- Skeletal malformations were not significantly different among groups.
- The percent per litter value of 13<sup>th</sup> full rib in the HD groups (41.7%) was non-significantly higher than control (29.3%) and within historical data.

PROJECT NO. \_\_\_\_\_  
 SPONSOR: GENENTECH INC  
 SPONSOR NO. SEN-119

STUDY OF B2036-PEG ON EMBRYO/FETAL DEVELOPMENT IN RABBITS  
 NUMBER OF FETUSES AND LITTERS WITH MALFORMATIONS - SUMMARY

DOSE GROUP:	FETUSES				LITTERS			
	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	89	128	102	94	16	16	15	16
CERPHALOCLE	1	1	0	1	1	1	0	1
SPINA BIFIDA	1	0	0	0	1	0	0	0
BUCKET HAIL	0	2	0	0	0	1	0	0
NUMBER EXAMINED VISCERALLY	89	128	102	94	16	16	15	16
HYDROCEPHALY	0	1	0	0	0	1	0	0
NUMBER EXAMINED SKELETALLY	89	128	102	94	16	16	15	16
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	3	0	2	2	2	0	1	2
RIB ANOMALY	0	0	3	0	0	0	1	0
STERNUM FUSED	1	0	0	0	1	0	0	0
STERNUM(C) MALALIGNED (SEVERE)	0	0	0	2	0	0	0	2
TOTAL NUMBER WITH MALFORMATIONS								
EXTERNAL :	2	3	0	1	2	1	0	1
SOFT TISSUE:	0	1	0	0	0	1	0	0
SKELETAL :	4	0	5	4	3	0	1	4
COMBINED :	6	4	5	4	5	2	1	4

1- 0 MG/KG/DAY    2- 1 MG/KG/DAY    3- 3 MG/KG/DAY    4- 10 MG/KG/DAY

DOSE GROUP:	MEAN LITTER PROPORTIONS OF VARIATIONS - SUMMARY % PER LITTER				DAY 29
	1	2	3	4	
NUMBER OF LITTERS EXAMINED SKELETALLY	16	16	15	16	
13TH SUPPLEMENTARY RIB(S)	MEAN 21.7 S.D. 23.13	MEAN 18.6 S.D. 17.35	MEAN 21.3 S.D. 18.76	MEAN 14.9 S.D. 15.08	
13TH FULL RIB(S)	MEAN 29.3 S.D. 28.26	MEAN 31.9 S.D. 26.97	MEAN 25.3 S.D. 22.72	MEAN 41.7 S.D. 32.26	
27 PRELUMBAR VERTEBRAE	MEAN 13.6 S.D. 19.57	MEAN 11.8 S.D. 17.15	MEAN 8.3 S.D. 8.26	MEAN 14.1 S.D. 17.91	
HYOID ARCH(ES) BENT	MEAN 4.9 S.D. 10.81	MEAN 2.7 S.D. 6.82	MEAN 3.3 S.D. 6.93	MEAN 3.3 S.D. 9.78	
STERNUM(C) IS AND/OR IS UNCLASSIFIED	MEAN 8.9 S.D. 13.31	MEAN 4.1 S.D. 9.69	MEAN 7.6 S.D. 18.61	MEAN 14.1 S.D. 27.43	
CERVICAL CENTRUM #1 UNCLASSIFIED	MEAN 0.0 S.D. 0.00	MEAN 0.0 S.D. 0.00	MEAN 1.3 S.D. 3.16	MEAN 0.8 S.D. 3.13	
HYOID BODY AND/OR ARCH(ES) UNCLASSIFIED	MEAN 0.0 S.D. 0.00	MEAN 0.0 S.D. 0.00	MEAN 0.0 S.D. 0.00	MEAN 0.8 S.D. 3.13	
STERNUM(C) WITH THREAD-LIKE ATTACHMENT	MEAN 5.8 S.D. 11.38	MEAN 0.8 S.D. 3.13	MEAN 1.8 S.D. 3.69	MEAN 3.6 S.D. 8.06	
25 PRELUMBAR VERTEBRAE	MEAN 0.6 S.D. 2.58	MEAN 0.0 S.D. 0.00	MEAN 1.9 S.D. 7.38	MEAN 0.0 S.D. 0.00	
STERNUM(C) MALALIGNED(SLIGHT OR MODERATE)	MEAN 2.2 S.D. 6.37	MEAN 2.1 S.D. 5.69	MEAN 0.0 S.D. 0.00	MEAN 0.9 S.D. 3.37	

1- 0 MG/KG/DAY    2- 1 MG/KG/DAY    3- 3 MG/KG/DAY    4- 10 MG/KG/DAY

NOTE: SIGNIFICANTLY DIFFERENT USING THE MANN-WHITNEY U TEST

Key Study Findings:

- No death in any group. No drug related maternal toxicity observed at any dose level.
- One female in low dose group aborted on gestation Day 21. No other abortions at any other group. ICH guidelines require between 16-20 females with litters.
- No change in maternal body weight, food consumption at any dose level.
- Slight erythema/edema at the injection site in the 1, 3 and 10 mg/kg/d B2036-PEG.
- No drug-related malformation or developmental variation in the fetuses at any dose group.
- The increase in postimplantation loss in HD group was 2-fold higher than control.
- Although it was not statistically significant, it is believed to be drug related since it was also observed in the HD group of the previous study (SEN-120).

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- The number of pups/litters with severely malaligned sternal vertebrae and/or unossified sternbrae were increased in pups of HD dams. This may be drug related since treatment caused thinning of the sternal and femoral bone in monkeys.
- B2036-PEG was not teratogenic. NOAEL was considered 3 mg/kg/day (3 times human exposure).

#### **Reproductive Toxicity Summary:**

In the first study, rabbits were treated with 1, 3 and 10 mg/kg from gestation Day 0 to Day 7. One female in the control group aborted on gestation day 21. All other animals survived to the scheduled necropsy on gestation day 21; no treatment related maternal toxicity was observed. Mean body weights, body weight gains and food consumption were unaffected by treatment at any dose level. Postimplantation loss was increased in the 10 mg/kg/day group (9X human dose). Whether the increased post-implantation loss is due to below normal IGF-1 levels or direct effect of B2036-PEG on GH receptors is not clear. Acromegalic patients, have significantly elevated IGF-1 levels that are returned to normal by B2036-PEG, in contrast to normal IGF-1 levels in rabbits that are significantly reduced by the treatment. If the postimplantation loss is via reduced IGF-1, a failure to sustain pregnancy in the 10 mg/kg/d group may not be of concern to humans or indicative of human results since rabbit IGF-1 levels were reduced to below normal levels. Intrauterine survival of the embryos in the 1 and 3 mg/kg/day groups was unaffected by test article administration. Based on the results of this study, NOAEL for maternal toxicity was  $\geq 10$  mg/kg/day and NOAEL for fetal toxicity is 3 mg/kg/day (3X human exposure).

In the second study, rabbits were treated with similar dose levels from gestation Day 7 to Day 20. One female in the 1 mg/kg/day group aborted on gestation day 23. No other abortions occurred in the study. No mortality or treatment-related clinical observations were noted at any dose level. Treatment-related dermal findings at the injection sites were noted in all pegvisomant groups and included slight erythema and/or edema. Food intake, mean maternal body weights, body weight gains and gravid uterine weights were unaffected by SC B2036-PEG. No treatment-related internal findings were observed at the scheduled necropsy in any treated group. Most parameters evaluated in the study were similar to control group values. Post implantation loss in HD group was nearly doubled compared to other groups. This was also noted in the early embryonic development study and is believed to be drug related. Malformations were observed in 6(5), 4(2), 5(1) and 4(4) fetuses (litters) from these fetuses respective groups and were considered spontaneous in origin. In conclusion, B2036-PEG administration had no maternal or developmental toxicity. Based on this study, B2036-PEG is not teratogenic. The NOAEL was 10 mg/kg/day for maternal toxicity and 3 mg/kg/day for fetal toxicity (3X human exposure based on body surface area).

#### **Reproductive toxicology conclusions:**

Early embryonic development and teratology studies were conducted in pregnant rabbits with pegvisomant at doses of 1, 3 and 10 mg/kg/day. There was no evidence of teratogenic effects associated with pegvisomant treatment during organogenesis. At 10 mg/kg/day pegvisomant (10 times human dose based on body surface area), a 2-fold increase in post-implantation loss was observed in both studies. Pegvisomant has been shown to suppress IGF-1 levels in rabbits at 10 mg/kg/day. The increase in post-implantation loss in rabbits could potentially be secondary to IGF-1 inhibition, since significant inhibition of IGF-1 below normal levels in dams could potentially interfere with normal embryonic development and growth in B2036-PEG sensitive animals. Fertility or teratogenicity studies were not conducted in rats since pegvisomant is not pharmacologically active in rodents. Overall, the doses used in these studies were not adequately high enough since no dose was associated with any sign of maternal toxicity in rabbits. The NOAEL for maternal toxicity was  $\geq 10$  mg/kg/day (10X human exposure) and 3 mg/kg/day for fetal toxicity (3X human exposure).

**Recommendations for future study:**

Since available data suggests potential for drug-related post-implantation loss or sternal skeletal malformations, even at the low dose levels evaluated. The sponsor should be requested to repeat the rabbit teratology study. The study should be conducted with a high dose level associated with some maternal toxicity. In addition, all reproductive toxicity studies were conducted with the Genentech product with 95% purity. The new product manufactured by \_\_\_\_\_ is less than 75% pure, therefore, the teratology study should be repeated with the final drug product.

**Labeling recommendations:**

Pregnancy: Pregnancy category B:

Early embryonic development and teratology studies were conducted in pregnant rabbits with pegvisomant at doses of 1, 3 and 10 mg/kg/day. There was no evidence of teratogenic effects associated with pegvisomant treatment during organogenesis. At 10 mg/kg/day pegvisomant (10 times human dose based on body surface area), a 2-fold increase in post-implantation loss was observed in both studies. Fertility or teratogenicity studies were not conducted in rats since pegvisomant is not pharmacologically active in rodents. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

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## **SPECIAL TOXICOLOGY STUDIES:**

**Study title: Dermal irritation potential of B2036-PEG after subcutaneous injection and effect of B2036-PEG on serum IGF-1 levels in the rabbit (SEN-115).**

B2036-PEG was administered to 3 female New Zealand rabbits at 3 mg/kg as subcutaneous injections on Days 0,1,2,6,7 and 8 of the study. A control group (3 females) was administered vehicle (buffer for compound reconstitution) following the same schedule. Blood was collected from each rabbit pre-dose and at 2 and 6 hours after dose administration on study days 2,3 and 8. Additional samples were taken at 26 and 30 hours after the last injection (2 and 6 hours on Day 9). Blood was processed to serum and stored frozen until analysis for IGF-1 concentrations. Body weights were determined on Days 0,6 and 9. On Day 9, after the last blood sample collection, the rabbits were euthanized and skin sections from the last two injection sites were excised for histopathologic examination. There were no deaths during the study period. There were no compound-related changes in rabbit body weights. Histopathological examination revealed microscopic findings of minimal infiltration at all injection sites including control animals. These findings were considered to be due to the route of administration. B2036-PEG did not produce histopathological evidence of dermal irritation.

**Study Title: Action of growth hormone antagonists in mice: discussion of immunogenicity data (SEN-122)**

Serum growth hormone antibodies were measured in mice using a non-GLP non-specific (<sup>125</sup>I-GH) RIA method. Mice received one of three purported growth hormone receptor antagonists or their pegylated analogs (G120K, B2034, B2036, G120K-PEG, B2034-PEG, or B2036-PEG). Serial dilutions of mouse sera were incubated with 0.05 M phosphate buffered saline and <sup>125</sup>I-hGH at 4 °C for 16-18 hours. Antibody/antigen complexes were precipitated using goat anti-mouse Y globulin. After centrifugation, supernatants were removed and the pellets were counted in a gamma counter. Both mice administered B2036-PEG had weak positive responses with 18 to 32% specific binding on Day 35 and titers of 1:250 and 1:300 for the two mice, respectively. No antibody response to B2036 was observed. It appears that B2036-PEG administration leads to a weak immune response in the mouse.

**Study Title: Antigenicity in rhesus monkey (SEN-102)**

Two monkeys were administered B2036-PEG daily at 0.2 mg/kg/day as SC injections for 14 days. Serum was taken at weekly intervals through 4 weeks after the last dose and analyzed for antibody production at \_\_\_\_\_ using the same methodology. One of the two monkeys administered B2036-PEG developed a weak positive response on Day 28 (2 weeks after the end of treatment). Binding was 12% and the titer was 1:220. It appears that B2036-PEG administration leads to a weak immune response in the monkey.

**Study Title: Study Title: Overview of Polyethylene Glycol Toxicity (SEN-211)**

In the early meeting with the agency, the possible of separation of PEG from B2036-PEG molecule and its toxicity was questioned. The PEG used in B2036-PEG is of approximately 5000 molecular weight. This section reviews publications where PEG 's of approximately 5000 Dalton were studied. In one report, PEG 's of 3000 to 60000 were administered IV to a variety of animals. The only adverse finding was the clumping of red cells in rabbits after the injection of a 10% solution of PEG 3350. PEG 6000 administration did not produce this effect. In another rabbit study, PEG 6000 was administered IV to rabbits at 1 g/day (6 days/week) for 5 weeks. Death in 1/9 rabbits occurred. In a dog study, PEG 3350 was given IV to dogs at dosages up to 90 mg/kg/day for 178

days. No adverse findings were reported nor was there evidence of PEG storage in phagocytic cells. The literature contains reports of findings after administration of PEG 's administered to rats and dogs by SC or IM injection. The only side effect observed was injection site irritation. Additionally, a review of the studies performed to support the registration of PEG 2000-bonded liposomal doxorubicin did not demonstrate any lesions attributable to PEG. Examination of the toxicity profile of PEG 's of molecular weight approximately the same as that used in B2036-PEG suggests that even if free PEG 5000 becomes systemically bioavailable during the metabolism of B2036-PEG, there would be no anticipated consequences of toxicological or biological significance.

This reviewer had examined other publication from Toxnet and Pubmed and found published data showing renal complication associated with topical drugs that had used polyethylene glycol as vehicle to treat burned patients. In these studies, renal tubular necrosis and fatal renal failure have been reported in burn patients repeatedly exposed to PEG-containing topical medications (McCabe et al, 1959; Bruns et al, 1982). A triad of high anion gap metabolic acidosis, hyperosmolality, and hypocalcemia has occurred in burn patients repeatedly exposed to PEG-containing topical medications; an increase in total serum calcium with a decreased or normal ionized serum calcium was noted (Bruns et al, 1982). Fatal renal failure occurred in some of these burned patients. PEG may be a human allergen or hapten. Anaphylaxis occurred in a 36-year-old man following ingestion of a multiple vitamin containing polyethylene glycol 8,000 and 20,000 (Kwee & Dolovich, 1982). Urticarial reactions have occurred following ingestion of PEG-containing colon lavage preparations (Brullet et al, 1992). PEG-containing lavage solution may exacerbate congestive heart failure in patients with severe left ventricular dysfunction and chronic renal insufficiency (Granberry et al, 1995). It was concluded from the results that alcohol dehydrogenase was the prime candidate enzyme for mammalian metabolism of polyethylene glycol.

In a animal study, Herold DA et al (Biochemical Pharmacology 38 (1): 73-6, 1989) investigated the enzymology of a fatal toxic syndrome that resulted from the absorption and subsequent oxidation of polyethylene glycol (PEG). The presence of organic acids of PEG in the blood of poisoned patients and in an animal model suggested that the metabolism of PEG involved sequential oxidations by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase. The polymer homologues of ethylene glycol from n = 1 to n = 8 were used as substrates. ADH catalyzed the oxidation of each of these PEGs. The oxidation of PEG was inhibited by the ADH inhibitor 4-methylpyrazole. With the exception of diethylene glycol, the Km decreased as the homologue number increased, and the Vmax decreased progressively through the series. The concentrations of PEG in the blood of poisoned humans and animals were 0.06 to 0.8 Km of ADH for all the PEG homologues above the triethylene glycol. These investigations established ADH as a candidate enzyme for mammalian metabolism of PEG and thus suggest that specific inhibitors of ADH may prove to be useful as tools to treat PEG poisoning.

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## Toxicology conclusions:

### 1. General Toxicity

In the 28-Day rhesus monkey study, doses of 0, 0.1, 0.3, 1 or 3 mg/kg/SC were administered every other day (14 days total). Animals in the 1 and 3 mg/kg/d dose had lower body weights (-15 and -12%) and HD monkeys had slight (8-16%) decrease in RBC, Hgb, and PCV. Monkeys dosed with 1 and 3 mg/kg had decreased alkaline phosphatase. The NOAEL was 0.3 mg/kg, which produced exposures 1/3 human dose based upon body surface area.

In the 3/6 month monkey study, animals were treated weekly with subcutaneous of 0, 1 and 3 mg/kg. There was body weight loss in both MD and HD animals. High dose animals had decreases in hemoglobin, packed cell volume and RBC counts. At doses  $\geq 1$  mg/kg, monkeys had lower serum phosphorus and lower serum ALP. The slight increase in BUN in HD animals returned to normal during recovery. Some of the major histological findings in HD (1/3 human dose) animals were "less prominent" islets of Langerhans, increase in fatty infiltration in colon, cecum, thymus; an increase in glycogen vacuolation in the liver; reduction in bone of the body of the sternum and reduction in trabecular bone in the femur. This was accompanied by diminution of the marrow of the femur, particularly in the femoral head of MD and HD animals. The bone effects were still observed in the recovery animals. Pegvisomant as expected reduced IGF-1 levels in monkeys but had no effect on prolactin. Insulin levels tended to be reduced but were variable. All treated animals (not controls) had increased frequency of injection site lesions. Overall NOAEL was 0.3 mg/kg on a weekly basis ( $\sim 3.6$  mg/m<sup>2</sup>/week). The doses studied are 1/23, 1/7 and 1/3 human therapeutic exposures based on body surface area. Overall, study was invalid since animals were dose weekly in comparison to daily dosing indicated for humans.

In the 3/6 month rat study, animals received daily doses of 0, 3, 10 and 30 mg/kg pegvisomant, SC. Only the highest dose in males significantly lowered body weight with no change in food consumption. Both MD and HD males had lower WBC, lymphocytes and platelet counts. Alkaline phosphatase levels decreased in a dose-dependent manner. The 30 mg/kg dose significantly increased liver and kidney weight in females but not in males. The increase in kidney weights correlated with nephropathy noted in female rats. Both 10 and 30 mg/kg/d dose (5 and 15 times human dose, respectively) significant increased the proteinuria in female rats. Proteinuria was present after drug free recovery suggesting renal damage in females was not readily reversible. As it has been reported in other toxicity studies, the thickening at the injection site increased in a dose-dependent manner. In clinical trials, the injection site irritation has been also observed in about 10% of subjects. Since this was rare in control, the injection site irritation is attributed to PEGylation. Injection site sensitivity with pegylated compounds has been reported in literature. The NOAEL was 3 mg/kg/day (1.5 X human dose based on body surface area).

2. Genotoxicity: Sponsor had carried out two genotoxicity test: Ames and Chromosomal aberration test. The Ames test was reviewed by Ron Steigerwalt. Under the test conditions, B2036-PEG did not cause reverse mutation of Salmonella or E.coli (negative Ames test). The chromosomal aberration test was also negative.
3. Reprotoxicity: A single dose-range finding study and two main studies (SEN-119, SEN-120) were conducted in rabbits using 0, 1, 3 and 10 mg/kg/day, SC. No male fertility studies were performed. Injection site inflammation and lesions was noted in rabbits as well. Pegvisomant, at doses as high as 10 mg/kg/day produced no maternal toxicity in rabbits. However, at 10 mg/kg/day pegvisomant appeared to double the incidence of postimplantation loss in rabbits. Based on these two studies, pegvisomant was not teratogenic in rabbits. The NOAEL for

maternal toxicity was 10 mg/kg/day (10X humane exposure) and 3 mg/kg/day for fetal toxicity (3X human exposure based upon body surface area).

4. **Carcinogenicity:** Sponsor has not performed the standard 2-year rodent bioassays. The agency had agreed to the submission of a carcinogenicity study during Phase IV development/post marketing period. In lieu of carcinogenicity studies, pegvisomant was given to mice bearing human meningioma tumors, 4 lines of human breast tumors, and murine colon tumors. In these in vitro and in vivo studies, pegvisomant did not increase tumor growth and in some cases appeared to reduce tumor growth rate. These studies appear to suggest pegvisomant could exert anti-tumor effects in patients with growth hormone responsive tumors.

**Overall Conclusion:** Sponsor had conducted two 6-month toxicity studies. In the 6-month monkey study, animals were dosed weekly and thus it was invalid. In addition, in the monkey study doses evaluated represented only fractions of human therapeutic exposures. Based on body surface area, the calculated daily dose of pegvisomant in monkeys were 1.7, 5.1 and 17.1 mg/m<sup>2</sup>. At the highest dose, the human exposure ratio (based on body surface area) was less than 1, thus providing no safety margins. In the 6-month rat study, the highest dose (30 mg/kg/day, 15X human exposure) significantly increased kidney weight with associated nephropathy and proteinuria in female rats. The NOAEL in female rats for proteinuria and nephropathy was 3 mg/kg/day (1.5X human exposure based on body surface area). Since the monkey study was invalid and data from rats suggests a possible nephropathy at only 1.5X human dose, a second valid study in a non-rodent animal model (monkey) should be performed to establish safety margins in monkeys. This is crucial since acromegalic patients will be receiving pegvisomant chronically on daily basis.

The two genotoxicity tests (Ames test, Chromosomal aberration assays) found no evidence of mutagenicity with pegvisomant. Reproductive studies were carried out only in female rabbits. In the both the early embryo development and organogenesis studies, pegvisomant at doses up to 10 mg/kg/day (10X human exposure) had no maternal toxicity and did not appear to be teratogenic. However, at 10 mg/kg/day (10X human exposure) pegvisomant increased post implantation loss. A standard 2-year rodent carcinogenicity study has not been performed.

Based on the renal findings in rats and no valid non-rodent toxicity study, this reviewer suggests a non-Approval action on the application. A 6-month non-rodent toxicity study should be performed prior to an approval decision. A repeat of reprotoxicity study in rabbits with doses high enough to cause some maternal toxicity should be performed. All the recommended toxicity studies should be conducted using drug product manufactured by the same facility and process as the to-be-marketed formulation. The protocol for a rodent carcinogenicity study should be submitted by the sponsor to the Executive for Carcinogenicity Assessment Committee for review as soon as possible. The carcinogenicity study can still be completed as phase IV commitment.

#### **APPENDIX/ATTACHMENTS:**

The following studies were reviewed by Ron Steigerwalt.

Studies reviewed by Ron Steigerwalt

Study #	Study Title	Page
SEN-201	Ames/ Salmonella-E.coli reverse mutation assay on B2036-PEG	<u>53</u>
SEN-103	Acute IV Toxicity in Mice	<u>57</u>
SEN-104	Acute IV Toxicity in CD-1 Mice	<u>59</u>
SEN-105	Acute SC Toxicity in Mice	60
SEN-106	14-Day IV Toxicity in CD-1 Mice	<u>62</u>
SEN-107	14-Day SC Toxicity in CD-1 Mice	<u>65</u>
SEN-002	The quantitative determination of B2036-PEG in mouse serum by RIA	<u>68</u>
SEN-102	Action of growth hormone antagonists in primates	<u>69</u>
SEN-203	Interaction between Somavert and regular or NPI insulin	<u>72</u>
SEN-108	B2036-PEG -28 day subcutaneous toxicity study in the rhesus monkey	<u>77</u>
SEN-115	Dermal Irritation Potential of B2036-PEG after SC Injection and Effect on Serum IGF-1 Levels in the Rabbit	<u>79</u>
SEN-109	B2036-PEG 26-Week Subcutaneous Toxicity Study in the Rhesus Monkey with an Interim Sacrifice after 13 Weeks Followed by an 8-Week Treatment-Free Period.	<u>85</u>

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