

4.3 General Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

4.3 General Toxicology Studies

Overview of Toxicology

Toxicology studies (all GLP except as noted) were performed with OP-1 using either the protein alone suspended in a diluent satisfactory for IV administration, as OP-1[®] Putty (a particulate mixture of OP-1, bovine Type I bone collagen, and carboxymethylcellulose [CMC]) or OP-1[®] Implant (a particulate mixture of OP-1 and bovine Type I bone collagen). Toxicity was assessed primarily by the SC (OP-1[®] Putty or OP-1[®] Implant) or IV (OP-1 protein alone) routes. Rodents and nonhuman primates were used for most of the toxicology studies. Nonhuman primates were used because OP-1 is a recombinant human protein. Guinea pigs and rabbits were used as standard species for dermal sensitization and developmental toxicology studies, respectively. The sequence of the mature protein is highly conserved among species. [Ozkaynak, 1991] In the TGF- β domain there is only one amino acid difference between mouse and human OP-1, and in the entire mature protein only 3 differences occur. Consequently, comparisons of experiments from different species and extrapolation of results across species including humans is more relevant.

Single- and multiple-dose toxicology studies were conducted with OP-1 and are summarized below:

- **Single-dose IV Studies** No adverse effects were identified in mice (NOAEL:10.7 mg/kg; 107 times the maximum human dose of approximately 0.1 mg/kg; based on 70 kg body weight) or rats (NOAEL: 3.5 mg/kg; 35 times the maximum human dose).
- **Multiple Dose IV Studies** In a multiple-dose 28-day IV toxicity study in male and female rats, a variety of mild but significant adverse events were noted at 3.5 mg/kg/day including lower body weights in males; minor changes in red blood cell (RBC), white blood cell (WBC) counts, albumin and globulin concentration, as well as in thymus weights in both males and females. These changes were small and not seen in the mid-dose group (NOAEL: 0.35 mg/kg/day; 3.5 times the maximum human dose given daily for 28 days). In female monkeys (1/group) treated IV, there was no evidence of systemic toxicity. (NOAEL: 1.0 mg/kg/day, 10 times the total human dose given 3 days a week for 4 weeks). Moderate to severe injection-site irritation and thrombophlebitis was observed in all drug-treated groups and the lesions were not reversed following an 8 week recovery period. Similar injection-site responses were also observed in rats, including ossification.

4.3 General Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

- **Implant Studies** No adverse events were noticed in implant studies (NOAEL: 0.41- 3.3 mg/kg; 4.1 to 33 times the maximum human dose) except for irritation at the SC site and some heterotopic bone, both expected in light of OP-1 pharmacology.

Genotoxicity (chromosomal aberrations, base-pair changes) was assessed in the CHO cell line (using OP-1[®] Implant) and Ames assays. At maximum concentrations, with and without S9, there was no evidence of clastogenicity or mutagenicity in either of these assays.

Dermal sensitization (Buehler Method) was assessed in male and female guinea pigs with OP-1[®] Putty. There was no evidence of cutaneous sensitization in these assays.

Embryo-fetal development was assessed in rats and rabbits in IV toxicity studies. In two rat studies (0.004 to 3.5 mg/kg; up to 35 times the human dose given daily for 12 days), OP-1 did not cause maternal or developmental toxicity when administered on Days 6 to 17 of gestation. In rabbits (0.004 to 0.4 mg/kg; up to 4 times the human dose given daily for 13 days), OP-1 did not cause maternal or developmental toxicity when administered on Days 6 to 18 of gestation.

Rabbits were intentionally immunized with human OP-1 in complete Freund's adjuvant and boosted several times in incomplete Freund's adjuvant; no changes were noted in dam or kits that could be attributed to OP-1. The only effects were differences in organ weights that may be attributed either to stress (maternal) or an underlying small difference in body weight between groups (kits).

Summaries of these studies are presented in Table 4-1 and in the text that follows.

4.3 Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

Table 4-1: Tabulated Summaries of GLP Toxicology Studies

Stryker Biotech Study ID	Title	Species	Test Article/ Lot No.	No. M/F per group	Dose & Dose Regimen	Fold Human Dose*	Parameters	Key Findings
Acute Toxicity – IV								
98-005	OP-1 acute IV toxicity test in mice	Mouse	OP-1 in 20mM acetate buffer; Lot AA7J007L	5 M 5 F	0–10.7 mg/kg single IV admin. Duration 15 days. Total OP-1 administered = 0–0.3 mg	107	Clin observations; body wt; gross findings at necropsy	No treatment-related toxicity
95-002	OP-1 acute IV toxicity in rats	Rat	OP-1; Lot AA4J011	5 M 5 F	0–3.5 mg/kg single IV admin. Duration 14 days. Total OP-1 administered = 0–0.7 mg.	35	Clin observations; body wt; clin pathology; gross findings at necropsy	No treatment-related toxicity
03-006	A study to determine the acute toxicity of Osteogenic Protein-1 (37C) following an IV injection into the Sprague Dawley rat	Rat	OP-1 in 5% lactose; Lot AA03J102	5 M 5 F	0–3.5 mg/kg single IV admin. Duration 14 days Total OP-1 administered = 0 to 0.6 mg.	35	Clin observations; body wt; clin pathology; necropsy, macro- & microscopic exam	No treatment-related toxicity
Multiple IV Dose								
00-003	Comparative 4-week toxicity study in cynomolgus monkeys	Monkey	OP-1 in 20mM acetate buffer; Lot AA7J020L	2 F (toxicity 4 wk); 1 F (8 wk recovery)	0–1.0 mg/kg per admin for 12 admins over 4 weeks Total OP-1 administered = 0–25.2 mg.	10X; 3 days/ wk for 4 wk	Clin observations; body wt; ECG, clin pathology; anti-OP-1 ELISA; OP-1 concentr by ELISA; sonograms; histopathology	No systemic toxicity noted. Local vasculopathy noted at all doses
96-001	28 Day IV toxicity study in rats administered OP-1	Rat	OP-1 in 20mM acetate buffer; Lot AA5J001	10 M 10 F	0–3.5 mg/kg/day for 28 days Total OP-1 administered = 0–25.3 mg.	35X for 28 days	Clin observations; body wt; clin pathology; histopathology	No treatment-related mortalities. Local toxicity based on gross findings of constricted white rings, microscopic findings of hyperplastic, and ossified cartilage at injection site. Male & female rats treated w/ 3.5 mg/kg/day for 28 days had lower body wts, lower serum albumin, lower red cell parameters, & increased serum globulin. Reduced ovary & adrenal wts also noted in

4.3 Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

Stryker Biotech Study ID	Title	Species	Test Article/ Lot No.	No. M/F per group	Dose & Dose Regimen	Fold Human Dose*	Parameters	Key Findings
								females.
Acute Toxicity – Implant								
97-011	OP-1 Putty – 14 day implant study in the rat	Rat	OP-1 Putty; Lot AH6A006H, CMC Lot AN6A001H	5 M 5 F	0–5.6 mg/kg with duration of 14 days Total OP-1 administered = 0–1.4mg.	56	Clinical observations and pathology Hematology Urine analysis Histopathology	No treatment related toxicities
07-012 Appendix 1a-1	Assessment of potential toxicity and toxicokinetics of OP-1 Putty in male and female New Zealand White Rabbits via a postero-lateral vertebral fusion model	Rabbit	OP-1 Putty H101167 (285-03-001) (OP-1) AN06A006 (collagen+ CMC) AG06A801 (Collagen)	3 M 3 F	0-3 mg/kg	30x	Clinical pathology and observations Hematology Histopathology Antibody determinations	No treatment-related toxicities
91-002	A study to determine the acute toxicity of the OP-1 Implant following subcutaneous implantation in laboratory rats	Rat	OP-1 Implant; Lot P002-1-M11-D1; Collagen matrix Lot BM11PD90-C1	5 M 5 F	0–3.3 mg/kg single SC administration with duration of 15 days Total OP-1 administered = 0–0.7 mg.	33	Clinical observations Body weight Gross findings at necropsy	No treatment-related toxicity
92-001	A study to determine localized inflammatory response to OP-1 Implant and the vehicle (collagen matrix) following subcutaneous implantation in Long Evans rats	Rat	OP-1 Implant; Lot P002-3,4-M15-D1, Collagen matrix Lot BM15 PD95-C1	5 M 5 F	0–0.35 mg/kg with duration of 22 days Total OP-1 administered = 0–0.9 mg	3.5	Clinical pathology Clinical observations Histopathology	No adverse toxic effects observed except for inflammatory response at implant site with both treatments.
98-024	OP-1 Implant 13 week toxicity study in rats with subcutaneous implantation	Rat	OP-1 Implant; Lot AH7A002L	15 M 15 F	0–6.9 mg/kg with duration of 13 weeks Total OP-1 administered = 0–1.8 mg.	69	Clin observations; body wt; hemato; clin chem; urinalysis; gross findings at necropsy; histopathology	Small body wt changes at 500mg; encrust & discolor of skin at implant site at 250mg (3.5 mg/kg) & 500mg (7.0 mg/kg). Heterotopic bone observed near inject site at 500mg.
95-001	Submucosal implantation	Hamster	OP-1 Implant	5 M	0–3.4mg/kg with duration of	34	Clin observations;	Very slight elev aspartate

4.3 Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

Stryker Biotech Study ID	Title	Species	Test Article/ Lot No.	No. M/F per group	Dose & Dose Regimen	Fold Human Dose*	Parameters	Key Findings
	test in the hamster 14 day time point		Lots AE4A002, AE4A003, AE4A004; Collagen matrix Lot AJ4A002	5 F	14 days Total OP-1 administered = 0– 0.34 mg.		body wt; gross necropsy; hematol/biochem;hi stopathology	aminotransferase & alanine aminotransferase noted in high-dose males only.
Genotoxicity								
96-004	Ames/Salmonella-E coli reverse mutation assay on OP-1	S. Typhimur ium (5 strains) E. coli (1 strain)	OP-1 Implant extracted with 0.9% saline; Lot AH5A004	Triplicate plates	69-6900 µg OP-1/plate +/- S9; positive & negative controls	n/a	Cytotoxicity; revertants	No cytotox or genetic tox at highest concentration, 6900µg/plate
97-001	Ames/Salmonella-E coli reverse mutation assay on OP-1 Implant/CMC	S. Typhimur ium (5 strains) E. coli (1 strain)	OP-1 Implant extracted with 0.9% saline Lots AH6A005H& AH6A006H; CMC Lot AN6A001H	Triplicate plates	69-6900 µg OP-1/plate +/- S9; positive & negative controls	n/a	Cytotoxicity; revertants	No cytotox or genetic tox at highest concentration, 6900µg/plate
98-009	OP-1 Implant chromosomal aberrations with CHO cells in vitro	CHO cells	OP-1 Implant extracted with 0.9% saline; Lot AH6A008H	Triplicate slides	0.004% to 1% of DMSO extracts; 0.35% to 90% of Ham's F-10 extracts ± S9; positive & neg controls	n/a	Cytotoxicity (monolayer cell counts) & chromosomal aberrations	No cytotoxicity or clastogenic activity
Cytotoxicity								
97-002	L929 agar overlay test for cytotoxicity in vitro	Mouse L929 cells	OP-1 Putty	Triplicate dishes	0.18 mg OP-1	n/a	Cytotoxicity	OP-1 Putty scored a 1 (slight tox) on USP23 reactive grade scale vs 4 by the pos control.
96-003	CHO mammalian cell cytotoxicity assay on OP-1 (OP-1 Implant)	CHO cells	OP-1 Implant; Lot U95002R	Triplicate plates	0.23–6900 µg/mL exposure for 5 hours	n/a	Cytotoxicity	Invalid: test system incompatible w/ OP-1 implant. known bio-incompatibility w/ CHO cells
Local Tolerance Sensitization & Hemocompatibility								
92-002	Dermal sensitization study of OP-1 Implant in guinea pigs (Buehler's technique modified)	Guinea pig	OP-1 Implant; Lot D7, Bulk Lot 008-1,2,3,4	Phase 1: 2M, 2F Phase 2:	Phase 1 Pre-induction: 0–1.19 mg/kg applied topically Total OP-1=0–0.4 mg	11.7-11.9 weekly for 4 weeks	Phase 1: Draize scale for skin irritation; body wt	OP-1 Implant is non-irritating & non-sensitizing

4.3 Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

Stryker Biotech Study ID	Title	Species	Test Article/ Lot No.	No. M/F per group	Dose & Dose Regimen	Fold Human Dose*	Parameters	Key Findings
				5M, 5F	Phase 2 Induction/Challenge 0–1.17 mg/kg applied topically 1/week for 4 weeks Total OP-1 = 0–1.3 mg		Phase 2: Draize scale; body wt; clin observation	
97-005	OP-1 Implant sensitizing potential in the guinea pig: (Epicutaneous maximization test)	Guinea pig	OP-1 Implant; Lot AH5A004, Bulk Lot AA4J022	10 M 10 F	0–0.87 mg/kg 8 topical applications over 32 days Total OP-1 administered = 0–2.8 mg	8.7 dosed 8 times in 32 days	Draize scale; body wt; clin observation	OP-1 Implant is non-irritating & non-sensitizing
97-006	OP-1/CMC Device sensitizing potential in the guinea pig: (Epicutaneous maximization test)	Guinea pig	OP-1 Implant; Lot AH5A004, CMC Lot AN6A001H	10 M 10 F	0–0.87 mg/kg 8 topical applications over 32 days Total OP-1 administered = 0–2.8 mg	8.7 dosed 8 times in 32 days	Draize scale; body wt; clin observation	OP-1 Putty (OP-1/CMC Device) is non-irritating & non-sensitizing
97-004	Assessment of the hemolytic properties of a test article: Direct contact and a saline test	Rabbit	OP-1 Implant; Lot AH6A004H, CMC Lot AN6A001H	Triplicate blood samples	.2 gram of test article/mL blood 0.7 mg OP-1 (for OP-1 Device)	n/a	Hemolysis	<5% hemolysis for OP-1 implant and CMC
Carcinogenicity								
00-004	OP-1 Implant: 104 week carcinogenicity study in rats with SC implantation w/ a 52 wk toxicity study	Rat	OP-1 Implant; Lots AH6A006H, AH6A005H	20-50 M 20-50 F	0–8.4 mg/kg Total OP-1 administered = 0–1.8 mg.	84	Clin signs; body wt; ophthalmoscopy; histol; hematol	Pleiomorphic sarcomas at implant site; bone formation at implant site; no systemic tox
Tumor Cell Proliferation								
02-007**	The effects of OP-1 on tumor cell proliferation	Mouse (tumor cell lines)	OP-1; Lots SOA001, AA00J009	8 M for in vivo studies	In one segment of the study tumor cell lines injected with 25 mg/kg OP-1	250	Clin exam; histology	Growth inhibition or no effect on 10/12 cell lines; prolif. in 2 osteosarcoma cell lines; No stimul. of appreciable tumor formation in 5 cell lines when co-incubated in nude mice; Growth inhib. of 1 cell line when co-admin. w/ cell line in nude mice
Reproductive & Developmental Toxicity								
98-004	OP-1 developmental toxicity range-finding study in rats	Rat	OP-1 in 20mM acetate buffer; Lot AA7J007L	6 F	0–3.5 mg/kg/day IV administration on gestation Day 6–17 Total OP-1 administered = 0–8.8 mg.	35 for 12 days	Clin observ; body wt; phys exam; compl. macroscop eval; Fetuses given gross extern exam & wt	No treatment-related maternal or developmental toxicity

4.3 Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

Stryker Biotech Study ID	Title	Species	Test Article/ Lot No.	No. M/F per group	Dose & Dose Regimen	Fold Human Dose*	Parameters	Key Findings
00-005	A study for effects of OP-1 administered on embryo-fetal development in rat	Rat	OP-1 in 5% lactose; Lot SOA001	24 F	0 to 0.4 mg/kg/day IV administration on gestation Day 6-17 Total OP-1 administered = 0-1.0 mg.	4.0 for 12 days	Clin observ; body wt; phys exam; compl macroscop eval; fetuses gross extern exam & wt & ½ each eval for soft tiss & skel defects by histol	No treatment-related maternal or developmental toxicity
Reproductive & Developmental Toxicity (continued from previous page)								
00-006	A study for effects of OP-1 administered on embryo-fetal development in rabbits	Rabbit	OP-1 in 5% lactose; Lot SOB001	23 F	0-0.4 mg/kg/day IV administration on gestation Days 6-18 Total OP-1 administered = 0-18.5 mg.	4.0 for 13 days	Clin observ; body wt; phys exam; compl macroscop eval; Fetuses had gross extern exam & wt & ½ each eval for soft tiss & skeletal defects by histology	No treatment-related maternal or developmental toxicity
99-004	125I-OP-1; Placental transfer in rat following single IV administration	Rat	125I-OP-1; Lot AA7J027L	3 F	3.8 mg/kg single IV administration on gestation day 18 Total OP-1 administered = 15.4 mg.	38	Autoradiography; tissue measure of radioactivity	Placental transfer of 125I-OP-1 to rat fetal tissue <1%.
04-002 04-010 04-011	Effects of OP-1 immunization on fetal development	Rabbit	OP-1; Lot AA8J005L	25-27 F	350µg OP-1 in complete Freund's adjuvant; 3 boosts w/ OP-1 (350 µg) in incompl Freund's adjuvant	n/a	Clin obs; body/ organ wts; compl macroscop eval; antibody measure	No definit. immuniz-assoc changes; all dams/kits (pre- & postnatal) developed IgM & IgG immunoreactivity

4.3 Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

Acute Intravenous Administration

OP-1 had no acute toxicity when administered as a single bolus IV injection to mice or rats at doses up to 10.7 mg/kg (Stryker Biotech Study 98-005) and 3.5 mg/kg (Stryker Biotech Studies 95-002 and 03-006), respectively. (See P060021; Appendix 2, pages 1898 and 1926 for complete study reports.)

Subacute Intravenous Administration

28-Day Monkey Study

In Stryker Biotech Study 00-003, (P060021; Appendix 2, page 2161), administration of OP-1 to female cynomolgus monkeys over a 4 week period resulted in systemic NOAEL of 1.0 mg/kg (the top dose used was 10 times the human dose given 3 times a week for 4 weeks) but was associated with local damage to the blood vessels used for compound injection. A local vasculopathy developed at all doses of OP-1, and was also observed in the control group receiving acetate buffer (though to a lesser extent). The lesions did not resolve during the 8 week recovery period. One possible instance of osseous differentiation at the injection site was noted at the 1.0 mg/kg dose. No systemic changes were noted.

28-Day Rat Study

In Stryker Biotech Study 96-001, (P060021; Appendix 2, page 2477), the toxicological effects of repeated IV administration of OP-1 were evaluated in rats. Daily administration of OP-1 in acetate buffer for 28 days resulted in a NOAEL for systemic toxicity of 0.35 mg/kg/day (3.5 times the human dose given for 28 days). Male and female rats treated with 3.5 mg/kg/day had lower body weights, lower serum albumin, lower red cell parameters, and increased serum globulin. Reduced ovary and adrenal weights were also noted in females. No NOAEL could be assigned for local toxicity due to the formation of macroscopic concentric and constricted white rings at injection sites corresponding to microscopic findings of hyperplastic and ossified cartilage at all doses. These latter observations are expected based on the pharmacology of OP-1.

Implant Studies

OP-1 was combined with bovine collagen and administered as an implant to rats, rabbits and hamsters as follows:

4.3 Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

In Stryker Biotech Study 97-011, (P060021; Appendix 2, page 2644), a 14 day rat study, 40-400 mg of OP-1[®] Putty (0.56 to 5.6 mg/kg of OP-1) was administered SC and no evidence of toxicity was observed at any of the doses.

In Stryker Biotech Study 07-012, (P060021/A011, Appendix 1a-1), a 35 day rabbits study, 3 mL of OP-1 Putty was divided equally and placed on either side of the vertebral column in a postero-lateral fusion model. The groups received Putty containing between 1-3 mg/mL OP-1 (0.75-3 mg/kg of OP-1). Spinal fusion was observed in all of the animals treated with OP-1 at any dose. No evidence of toxicity was observed at any of the OP-1 doses.

In Stryker Biotech Study 07-012, (P060021/A011, Appendix 1a-1), a 35 day study, OP-1[®] containing 0.25-1.0 mg/kg of OP-1 was administered SC with the same results.

In Stryker Biotech Study 91-002, (P060021; Appendix 2, page 2929), a 15 day study was conducted with OP-1[®] Implant (0 to 3.3 mg/kg of OP-1) with the same results.

In Stryker Biotech Study 92-001, (P060021; Appendix 2, page 3071), a 22 day rat study, OP-1[®] Implant (0.35 mg/kg of OP-1) was administered and no sign of toxicity was noted except for implantation-site irritation.

In Stryker Biotech Study 98-024, (P060021; Appendix 2, page 3244), a 13 week rat study, OP-1[®] Implant (1.7 to 6.9 mg/kg of OP-1) was administered with minimal toxicity. The only abnormalities noted were a small decrease in body weight in each sex and the formation of focal SC bone at the implant site in the 500 mg group (containing 6.9 mg/kg OP-1).

In Stryker Biotech Study 95-001, (P060021; Appendix 2, 3457), a 14 day submucosal implantation (cheek pouch) study was conducted in hamsters using 10 mg of collagen matrix containing up to 3.4 mg/kg of OP-1. Elevation of aspartate aminotransferase and alanine aminotransferase were noted in the high-dose males, but not in females or at other doses. In summary, in the acute implantation studies, NOAEL varied between at least 3.5 and 69 times the maximum human dose.

Genotoxicity

OP-1[®] Implant and OP-1[®] Putty were each negative for genetic toxicity in the Ames/Salmonella-E. coli reverse mutation assay in Stryker Biotech Studies 96-004 and 97-001, respectively (see P060021; Appendix 2, page 3501 and 3524), and had no

4.3 Toxicology Studies

(Content from P060021/A011 Section IV, Preclinical: Section 4, page 42-67)

clastogenic activity in the CHO chromosomal aberration assay in Stryker Biotech Study 98-009, (see P060021; Appendix 2, 3553).

OP-1 Local Tolerance

***In Vitro* Cytotoxicity**

In Stryker Biotech Study 97-002, (P060021; Appendix 2, page 3600), OP-1[®] Putty (50 mg; about 0.175 mg of OP-1) was tested in an L929 Agar Overlay Assay and was only slightly cytotoxic (1 on a scale of 1 to 4, indicating some malformed or degenerated cells were present). In contrast, latex, the positive control yielded a rating of 4 (cytotoxicity zone extends greater than 1 cm beyond the specimen but does not involve the entire dish). A similar study was done with CHO studies that was judged invalid due to a known incompatibility with the cell line (Stryker Biotech Study 96-003; P060021, Appendix 2, page 3621)

Sensitization and Hemocompatibility Studies

In Stryker Biotech Study 92-002 (P060021; Appendix 2, page 3637), dermal sensitization (Buehler Method) was assessed in male and female guinea pigs with OP-1[®] Implant. Up to 100 mg of implant (0.35 mg/kg of OP-1) was applied to an absorbent pad and affixed occlusively to the back of each animal. There was no evidence of cutaneous sensitization in these assays. OP-1[®] Implant and OP-1[®] Putty were also inactive in the epicutaneous maximization test (Stryker Biotech Study 97-005 and 97-006; P060021; Appendix 2, pages 3772 and 3824).

In Stryker Biotech Study 97-004, (P060021, Appendix 2, page 3879), OP-1[®] Implant and CMC were each negative in a standard hemocompatibility test at a concentration of 0.2 g test article/mL rabbit blood indicating that the device does not cause erythrocyte lysis to any significant extent.

References

Ozkaynak E, Schnegelsberg PN, Oppermann H. Murine osteogenic protein (OP-1): high levels of mRNA in kidney. *Biochem Biophys Res Comm.* 1991;179:116–123.