4.4.1 Tumor Biology

Carcinogenicity

Stryker Biotech Study 00-004, (P060021, Appendix 2, page 3903) was conducted to explore the potential carcinogenicity and toxicity of OP-1® Implant. Five (5) groups of 50 male and 50 female Sprague-Dawley rats were implanted SC with OP-1® Implant at dose levels of 0,125, 250, or 500 mg (up to 8.4 mg/kg OP-1) to evaluate carcinogenicity over a 104 week time course. In addition, 5 groups of 20 male and 20 female Sprague-Dawley rats were implanted with OP-1® Implant at dose levels of approximately 0, 125, 250, or 500 mg (up to 8.4 mg/kg OP-1) to evaluate implant toxicity. The highest dose used in these studies was chosen based on the highest feasible dose in the 13 week implant study in rats (Stryker Biotech Study 98-024; see Section 4.4). All animals were monitored during the entire in-life portion of the study for clinical signs of toxicity, body weight change, and food consumption. Ophthalmoscopy was carried out on all toxicity study animals before implantation and on all surviving control and high-dose toxicity study animals during Week 52 of the study. Blood and urine samples were taken for laboratory investigations from 10 male and 10 female toxicity study animals per group during Weeks 26 and 52. Blood samples were taken from all surviving carcinogenicity study animals for hematology during Weeks 103 or 104.

At 52 weeks (toxicity study) and 103/104 weeks (carcinogenicity study) after implantation, all surviving animals were euthanized and necropsied. All animals were given a detailed postmortem examination during which all major organs were weighed (toxicity study animals only) and a full set of tissues (including the implantation sites) preserved in fixative. Histological examination of these tissues was done on all control and high-dose animals and on all animals that died before the end of the study. Implantation sites were also evaluated in the low- and intermediate-dose groups.

Very few animals died before the end of the toxicity study, and nearly half of the animals died in the longer carcinogenicity study. Animals that received OP-1 implantation showed no difference in food consumption, water consumption, or ophthalmoscopic parameters. In addition, all laboratory values were normal. In postmortem examinations, there were no intergroup differences in either sex after 52 weeks of dosing. Masses were found at the implantation site of the majority of animals in all groups implanted with OP-1® Implant. The amount of circumscribed SC ossification occurring at the implantation site in animals of all groups treated with OP-1® Implant correlated with OP-1® Implant levels. This finding was expected given the

osteoinductive nature of OP-1 when combined with an osteoconductive matrix and implanted in a permissive site such as the SC space.

Pleomorphic sarcomas were observed at the implantation site in both the toxicity study and carcinogenicity study animals. The tumors were associated with the ossification and fibrous tissue that formed as a foreign body in SC tissues at all doses of OP-1. Diverse tumors of this type are commonly associated with the SC implantation of materials such as metals, glass, and polymers in rodents. Solid state carcinogenicity was first characterized in the 1950s and is referred to as the Oppenheimer effect.[Oppenheimer, 1955; Brand, 1975] The similarities between the characteristics of solid state carcinogenesis and the tumors observed in the present study include the following:

- **Tumor cell heterogeneity**. Although tumors associated with solid state carcinogenic processes are usually of mesenchymal origin, there is no correlation between a biomaterial group and a specific histologic cell type. [Brand, 1975] Malignant fibrous histiocytomas are very common followed by pleomorphic sarcomas. Examples of cell types observed include (but are not limited to) hemangiosarcoma, angiosarcoma, leiomyosarcoma, rhabdomyosarcoma, and fibrosararcoma. In the present study, fibrosarcoma, chondrosarcomas, histiocytic sarcoma, osteosarcoma, and hemangiosarcoma were observed along with sarcomas of a mixed cell type.
- **Fibrous tissue capsule**. A fibrous tissue capsule is prerequisite condition for solid state carcinogenesis. [Brand, 1975] The results from a 14 day study with OP-1® Implant have shown that there is clear evidence of capsule formation and fibrosis surrounding the implantation site (Stryker Biotech Study 97-011) that is characteristic of a foreign body response. Also, the results from the 104 week study showed an increased incidence in the presence of fibrotic activity at the implantation site.
- **Relationship between tumor induction and size of the stimulus**. Studies have demonstrated a relationship between implant size and the propensity to develop tumors. [Kirkpatrick, 2000; Stinson, 1964] A significant direct correlation was obtained in the present study between the size of an implant-related mass at 6 months and the tendency to form tumors. Moreover, within each OP-1 dose group for both males and females (with the exception of the low-dose male group), there was a statistically significant increase in the size of the implant mass at 6 months in subgroups of rats with tumors (Stryker Biotech Study 06-003). In the low-dose male group, there was a difference between the average mass sizes but the number of total rats with tumors was very low

(N=4). These data infer that the formation of sarcoma was due to a mechanical phenomenon dependent on the size of the implant mass irrespective of the dose of OP-1 administered.

- **Relationship between latency period and implant size**. There is a tendency for larger implants to shorten the latency period to the development of tumors in solid state carcinogenicity. In the present study, results indicate that the tumors appeared more quickly in the animals with larger implant masses (Stryker Biotech Study 06-003).
- **Presence of the implant mass for the duration of the latency period**. In solid state carcinogenesis the materials are present for the entire latency period. In this study, all tumors were associated with persistent bony masses. In contrast, some rats developed an implant mass at an early time point that later resolved by the time of necropsy. None of the animals in the latter group developed sarcomas.

After treatment with OP-1® Implant, the observed sequence of events, correlation with implant mass size, and tumor cell types (including heterogeneity within a single tumor) are identical to the characteristics that are described in classical solid state carcinogenesis studies (Oppenheimer effect).

Solid state carcinogenesis has not been observed in humans despite the broad array of substances and shapes routinely used in implantation procedures. Brand and Brand collected specimens of human foreign-body reactive tissues and evaluated the presence of preneoplastic cells. They were unable to detect any precancerous cells in any of the 27 specimens screened (implants were in place for 1 to 19 years). [Brand, 1980] Similarly, using oncogene localization techniques, the capsules around breast implants were examined in 24 patients. No evidence of sarcoma was detected in any of the implants after implantation for up to 24 years. [Schwalbe, 2003] In addition, autologous bone has been used for many orthopedic applications without evidence of a cancer risk.

No other neoplastic or non-neoplastic findings were detected at sites other than the implantation site. Likewise, no bone formation was detected in any organ investigated in any dose group.

See P060021; Appendix 2 for complete study reports.

Effects of OP-1 on Tumor Cell Proliferation

In response to the carcinogenicity study results presented above, FDA requested that the following studies be performed. This request was received in a written correspondence from August 10, 2001 following the initial review of HDE H010002 for OP-1® Implant. In the

correspondence, FDA agreed that the transformed cell growth observed at the site of OP-1 implantation most likely occurred due to the Oppenheimer solid state carcinogenesis phenomenon. However, it was felt that the additional studies, described below in three phases, were needed to better assess the potential for OP-1 to enhance the growth of pre-existing tumors.

In Stryker Biotech Study 02-007, (P060021, Appendix 2), the effect of OP-1 on cell proliferation *in vitro* was evaluated in a non-GLP study using a panel of 12 human tumor cell lines (SAOS 2, U2 OS, MG 63 and G 292, osteosarcoma; MCF and MDA MB 231, breast carcinoma; LNCaP and PC 3, prostate; PANC 1, pancreatic; G 402, kidney; A 549, lung; and SW 579, Thyroid. In addition, a series of *in vivo* studies were conducted to assess whether the effects seen *in vitro* are also observed in animals. These studies were conducted in 3 phases and are summarized below.

Phase 1 (OP-1 Receptor Expression *In Vitro*)

The first study was conducted to test a panel of 12 human tumor cell lines for the expression of Type I (ActR-I, BMPR IA, BMPR IB) and Type II (BMPR II) OP-1 receptor mRNA by northern blot. The results showed that all 12 cell lines expressed mRNA for the OP-1 receptors investigated. The level of mRNA expression, however, varied widely among the different cell lines, even among tumor cells originating from the same tissue. Because all 12 cell lines were positive for BMP receptor expression, they were all included in subsequent studies to determine the effects of OP-1 on tumor cell proliferation.

Phase 2 (Cell Proliferation *In Vitro*)–Initial Study

Cellular proliferation was measured *in vitro*, following exposure to varying concentrations of OP-1. Cellular proliferation was assessed by both [3H]-thymidine uptake and tetrazolium salt metabolism. The study results show that the effect of OP-1 on cell proliferation varied widely depending on the cell line tested, and in some cases on the assay used. Cell growth induction with a characteristic logarithmic dose response curve was observed only for U2 OS cells. In this case, exposure to OP-1 resulted in a 4 fold increase in the rate of DNA replication. This effect was not reflected, however, in the tetrazolium assay results. Different outcomes from these two assays may be due to the fact that DNA replication was measured for only 24 hours post OP-1 treatment. In this short window, cells may have progressed to S phase without completing cell division. As a result, the amount of metabolizing enzymes present would still reflect the initial number of cells. In general, [3H]-thymidine uptake is more useful for assessing cytostaticity whereas the tetrazolium assay is more useful for measuring cytotoxicity. Often these measures are not identical.

The effects of OP-1 on the other cell lines included a slight stimulation of proliferation for SaOS 2 (osteosarcoma), PC3 (prostate), and MB 231 (breast) and cell growth inhibition for SW 579 (thyroid), PANC1 (pancreas), G-402 (kidney), and A 549 (lung). The latter effect is more consistent with the known function of OP-1 as an inducer of cell differentiation with an associated cell growth inhibition effect.

Phase 2 (Cell Proliferation)–Supplemental Study

In the initial study described above, the effect of OP-1 on cell proliferation was measured using [3H] thymidine incorporation and a tetrazolium colorimetric assay. Data generated by the 2 assays were comparable for 8 of the 12 cell lines studied. For the remaining 4 cell lines, however, the data produced by the 2 assays differed significantly. In order to resolve these differences, an additional study was conducted to further examine the effects of OP-1 on cell proliferation. In this follow-up study, cell proliferation was assessed by cell counting under light microscopy. Analysis of the data obtained by the 3 experimental methods indicated that the results of actual cell counts were consistent with those of [3H] thymidme incorporation. Although the absolute extent of the effects differed, the data showed that OP-1 (particularly at 100 µg/mL) significantly inhibited the growth of MB 231, LNCaP, A 549, SW 579, MG 63 and G 292. In contrast, OP-1 exerted positive effects on the proliferation of U2 OS and SaOS 2 cells. However, a dose dependent effect was only noted for U2-OS.

In summary, the results of these studies show a positive effect of OP-1 on the *in vitro* growth kinetics of 2 osteosarcoma cell lines (U2 OS and SaOS2). This effect was dose dependent for U2-OS. At high concentrations (50 to 100 µg/mL) however, OP-1 treatment inhibits cell growth of the majority of the remaining cell lines tested (including the other 2 osteosarcoma cell lines).

Phase 3 (Tumor Induction in Nude Mice *In Vivo*)

Initially, the ability of the 12 cell lines to form solid tumors following SC injection in nude mice was evaluated (Phase 3, Part 1). The cells were incubated for 24 hours with OP-1 and were washed prior to injection. Of these, 5 cell lines formed tumors in at least one of the animals injected: 4/4 for A 549, 3/4 for PC 3, and 1/4 for PANC 1, MG63 and MCF 7 cells. PC 3, MG 63, A549, G 402, and SaOS 2 were used in subsequent studies where the effect *in vivo* effect of OP-1 was assessed (Phase 3, Part 2). In the case of A 549 and PC 3, no significance differences in growth kinetics or cell morphology were observed between the control (untreated cells) and the OP-1 treated groups. Pretreatment of the remaining 3 cell lines (MG 63, G 402 and SaOS-2) with OP-1 resulted in heterotopic bone growth at the injection site. It is possible that some residual OP-1 was transferred into the injection site due to an inadequate cell wash step. In a

subsequent control study (Part 3 of Phase 3 summarized below) with OP-1 alone, significant bone formation also occurred after SC injection. In some of the animals (1/8 for MG 63, 2/8 for SAOS 2, and 6/8 for G 402), bone nodules were associated with small tumor cell foci. Given the small size of the foci, it is likely that these cells were entrapped by the new bone that was formed. No metastases were noted for any of these cell lines. These 3 cell lines did not induce detectable tumor foci *in vivo* when treated with solvent alone.

Finally, in Part 3 of the Phase 3 study, the effect of injecting OP-1 alone, or in combination with untreated PC 3 cells directly in the flank of nude mice was assessed. Three (3) groups were included in this study: OP-1 alone (N=8), PC 3 cells alone (N=8), and OP-1 + PC 3 cells (N=8). Body weights and mass volume were recorded every 3 to 4 days for 49 days post-injection. At this time point, animals were sacrificed, the masses collected and processed for histological analysis. OP-1 appeared to weakly inhibit the formation of tumors. As in the previous studies, local heterotopic bone was observed at the injection site. This effect is consistent with the known bone growth promoting activities of OP-1.

In summary, the present results describe the effects of OP-1 on the proliferation and tumor-forming activity of a number of cancer cell lines. OP-1 either inhibited or had no effect on proliferation in 10 of 12 cell lines. In 2 cell lines, OP-1 stimulated proliferation in a dose-dependent manner. Both of these cell lines are osteosarcoma cell lines, so the effects of OP-1 are not surprising. In the *in vivo* studies, OP-1 was either pre-incubated or co-injected with several of the cell lines. In the pre-incubation study using cell lines that otherwise did not induce tumors in nude mice, the primary effect noted was the formation of bone nodules at the injection sites. In some of the nodules, small tumor foci were found, presumably due to entrapment within the bone matrix. There was no evidence that the OP-1 induced additional tumor expansion within the foci. In the co-injection study utilizing a cell line (PC 3) capable of forming tumors in nude mice, mixing the tumor cells with OP-1 appeared to have a mildly inhibitory effect on subsequent tumor formation. This effect is consistent with published results that indicate OP-1 inhibits proliferation in certain prostate tumor cell lines. [Miyazaki, 2004] Also, in results from a recent study, OP-1 did not promote PC-3 cell migration or invasion, while BMP-2 and -4 had the opposite effect.[Felly, 2006] In conclusion, the results presented in the report appear to have no adverse implications for the development of OP-1.

References

Brand BG, Buon LC, Brand I. Foreign body tumorigenesis induced by glass and smooth and rough plastic: comparative study of preneoplastic events. J Natl Cancer Inst. 1975;55:319–322.

Brand GK, Brand I. Risk assessment of carcinogenesis at implantation sites. Plastic and Reconstruction Surgery. 1980;66:591–594.

Kirkpatrick CJ, Alves A, Kohler H, et al. Biomaterial-induced sarcoma: a novel model to study preneoplastic change. Am J Pathol. 2000;156:1455–1467.

Schwalbe M, Sanger J Eggers R, et al. Differential expression and regulation of bone morphogenetic protein 7 in breast cancer. Int J Oncol. 2003;23:89–95.

Stinson NE. The tissue reaction induced in rats and guinea pigs by polymethyl methacrylate and stainless steel. Br J Exp Path. 1964;45:21–29.

Miyazaki H, Watabe T, Kitamura T, Miyazono K. BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells. Oncogene. 2004;23:9326–9335.

Felly, BT, Krenek, L, Liu, N et al. Overexpression of noggin inhibits BMP-mediated growth of osteolytic prostate cancer lesions. Bone 2006; 38:154-166.