

sites appeared clinically normal. Injection site reactions, after subsequent doses at intervals of 30 or 60 days, were similar to those noted after the first dose. The reactions at sites receiving multiple injections also gradually diminished and the sites were essentially normal in appearance by 2 to 4 months after the last dosing.

For all sacrifices up to Day 134 (2 to 6 weeks after the most recent treatment), residual test material was grossly visible at the local injection sites within the muscle and in some cases in small raised areas on the adjacent superficial muscle surface. The residual material within some injection sites was firm and well-defined while in others it was more diffuse and viscous. As the study progressed, less residual material was present. Adhesions were present in the subcutaneous region between the skin and superficial muscle surface at the injection sites in some animals (261/o). Tissue adhesion was rarely evident at the 6-month post-dose sacrifices (Days 210, 240 and 301).

Microscopically, residual test material was readily identified in injection site sections as retractile material. The residual material was progressively degraded, in that, by the 6-month post-dose sacrifices, significantly less foreign material was present compared to sites from earlier sacrifices.

The predominant histopathological findings consisted of an inflammatory reaction with fibrosis. For most sites evaluated approximately 2 weeks after dosing, chronic active inflammation (moderate to severe) was observed and was characterized predominantly by macrophages and multinucleated giant cells as well as neutrophils, eosinophils and lymphoplasmacytic cells. A component of granulomatous inflammation (minimal to severe) was also present in occasional sites at 2 weeks post-dose but this change was more obvious in sites examined at longer intervals after dosing. At the Day 44 sacrifice for Group 1 animals, chronic active inflammation was observed at sites from the left leg (2 weeks post-dose), whereas granulomatous inflammation was seen in the right leg sites (6 weeks post-dose). In Group 4 animals sacrificed at Day 134, 2 of 4 animals presented with primarily granulomatous inflammation, whereas the remaining exhibited chronic active inflammation. Minimal to moderate degeneration/regeneration of skeletal muscle was also observed at some injection sites primarily at earlier sacrifices. Minimal to moderate edema was noted in some, but not all injection sites at the 2-week post-dose sacrifices (Days 18, 44, 74 and 134) and was slightly more prominent with multiple dosings. Occasional minimal injection site hemorrhage observed in a few animals was attributed to the dosing procedure.

The recovery sacrifices conducted 6 months after the last dosing and up to 10 months after the first dosing (Group 1), demonstrated that polymer degradation was progressive and that it was accompanied by evidence of reversal of local inflammation and fibrosis. At the recovery time points, chronic active inflammation was no longer present and a lower incidence and/or severity of granulomatous inflammation was observed. Similarly, minimal to moderately severe fibrosis, most apparent at earlier sacrifices had diminished significantly by the time of the recovery sacrifices.

Study title: Investigative Local Tolerance Study of Medisorb® Naltrexone in Dogs Following Intramuscular Administration / ~~6403-123~~ Sponsor Reference No: AT-21-06)

In a previous study with beagle dogs (Alkermes Reference AT-21-05), an injection site reaction (a localized swelling) was clinically evident following intramuscular administration of Medisorb Naltrexone. In other species (rabbits, monkeys and humans), Medisorb Naltrexone did not produce similar reactions following intramuscular administration. The purpose of this investigative study was to further assess the injection site response following intramuscular administration of Medisorb Naltrexone in dogs. The study was designed to assess the following: 1) the effect of dose on the severity of injection site reactions to Medisorb Naltrexone, and 2) the effect of using a different lot of Medisorb Naltrexone to that used in study AT-21-05 on the severity of injection site reactions.

Four male and four female purebred beagle dogs approximately 7 to 10 months of age and 6.2 to 11.5 kg at study initiation, were utilized in the study. Medisorb Naltrexone was suspended in 1.2 mL of Medisorb Diluent just prior to administration (within approximately 5 to 10 minutes). The nominal microspheres concentration was approximately 280 mg/mL. For Group 3 (4 mL dose volume), three vials were pooled to deliver each microsphere dose. For Groups 1 and 2 (1 mL dose volume), one vial was used for each dose.

All animals survived to the scheduled necropsy. There were no adverse body weight changes during the study. Clinical effects were limited to injection site reactions, as described below. Following intramuscular injection of Medisorb Naltrexone, a clinically evident injection site reaction (a localized swelling) developed. This reaction was similar to that observed in the AT-21-05 dog study. The injection site reactions were dose related in that the onset of the reaction was earlier, the size of the swelling was larger and the reaction was more persistent in animals that received 4 mL compared to those that received a 1 mL dose. Swelling was noted in both Group 3 animals, beginning on Days 2 or 4 and was still observed on Day 15. Serosanguineous discharge was also present for one Group 3 animal (#H3 8505) on Day 10. Swelling was present in most animals in Groups 1 and 2 ranging between Days 7 and 15. Reddened skin was noted at a saline control injection site for one Group 3 animal on Day 7. No other findings were apparent for saline control injection sites.

At necropsy, residual test material was observed at most injection sites, either within the muscle and/or as a raised area in the adjacent subcutaneous region. Some injection sites exhibited a pale fluid filled pocket surrounding the test material within the muscle. In addition, tissue adhesion was present in the subcutaneous region between the skin and just above the muscle at the injection sites of some animals. Macroscopically, a greater amount of residual test material was observed for sites which received 4 mL compared to those that received 1 mL. No macroscopic findings were noted for saline control sites.

Study title: Investigative Local Tolerance Study of Medisorb® Naltrexone in

Rabbits Following Intramuscular Administration 6403-122 Sponsor
Reference No: AT-21-07)

In a previous study with beagle dogs (Alkermes Reference AT-21-05), an injection site reaction (a localized swelling) was clinically evident following intramuscular administration of Medisorb Naltrexone. In other species (rabbits, monkeys and humans), Medisorb Naltrexone did not produce similar reactions following intramuscular administration. The purpose of this investigative study was to assess the local tolerance for Medisorb® Naltrexone when administered intramuscularly as a single dose or as two consecutive doses to rabbits. Three male and three female Hra:(NZW)SPF Rabbits, approximately 18 to 20 weeks of age and 2.5 to 3.0 kg at study initiation, were utilized in the study. Medisorb Naltrexone was suspended in 1.2 mL of Medisorb Diluent just prior to administration (within approximately 5 minutes). The nominal microsphere concentration was approximately ng/mL and the target dose volume for each intramuscular injection was approximately 3.0 mL (a maximum intramuscular volume in rabbits). Two vials were pooled to deliver a single dose. The nominal dose per injection for Medisorb Naltrexone was approximately ng (approximately 294 mg of naltrexone). All animals were clinically normal throughout the study and survived to the scheduled necropsy. There were no adverse effects on body weights during the study. Intramuscular injection of Medisorb Naltrexone or Medisorb Diluent did not result in any adverse reactions at the local injection sites. A slight raised area was noted one hour after dosing for one animal each from Group 2 on Day 1 and Group 3 on Day 8. The raised areas in both animals did not persist and injection sites were clinically normal one day after dosing. No other test or vehicle control related injection site findings were noted clinically. The macroscopic findings were limited to the intramuscular injection sites. At necropsy, a light focus interpreted as residual test materials was observed at all intramuscular Medisorb Naltrexone dose sites. Findings for all injection sites consisted of a single, firm and tan area within the muscle. One or two distinct firm tan areas were observed within the muscle. A small light focus area of residual material on the surface of the muscle was also observed. There were no macroscopic findings for any of the vehicle control treated sites. There were no other macroscopic findings reported.

Genetic toxicology:

The Sponsor is referencing the genetic toxicology data that was evaluated for the Revia NDA and described in the approved label. The Sponsor also submitted their evaluation of the genetic testing results and copies of the published literature that served as the basis of the Revia genetic toxicology submission. Of the genetic toxicity studies conducted with naltrexone, only the Drosophila sex linked recessive lethal assay was positive. Specifically, a concentration of 10 mg/ml naltrexone was found to consistently increase recessive lethal frequency of the experimental groups 2-3x over their controls. Naltrexone was administered at 7.0 x 10.0 mg to the flies either by feeding (6 tests) in 10% sucrose or by injection (2 tests) in saline. Up to four broods, were examined covering several stages of spermatogenesis; sperm (Brood I), spermatids (Brood II), spermatocytes (Brood III), and spermatogonid (Brood IV). The results showed a

consistent positive response at 10 mg/ml in the post meiotic cells. Both injection and feeding studies produced similar responses at this concentration.

Naltrexone was also tested in in vivo chromosome alteration studies with both somatic cell and germinal cell risk assessments completed. Doses of 90, 300, and 900 mg/kg were administered by gavage to rats. Animals were killed at 6, 24, and 48 hrs, and after an acute exposure and at 6 hrs after a 5 day subchronic exposure. No evidence for either clastogenicity or mitotic inhibition was obtained.

Naltrexone was evaluated for its ability to induce reciprocal translocations in mouse sperm cells using the Heritable Translocation Assay (HTA). Male mice were treated by oral gavage with 103, 343, and 1030 mg/kg/day for seven weeks. One hundred F₁ male progeny from crosses of the exposed males to unexposed females were mated sequentially to three sets of females and the embryos scored for evidence of semisterility. All semisterile and sterile males were examined cytologically. No confirmed translocation carriers were found in the control or treatment groups with the exception of TEM, the positive control. No non-disjunction in the experimental animals was found. Inconsistency was seen in the in vitro cytogenetic evaluations, in the metaphase analysis. One study reported positive results in lymphoblast cells but a second study reported negative results in CHO cells. Similar inconsistency was observed in the Sister Chromatid Exchange assay, positive results in long-term human lymphoblast cells but negative results were reported in CHO cells. Anaphase analysis in vitro was the only consistent observation of a positive nature.

Secondary DNA repair tests with *E. coli* and WI-38 cells indicated weak non specific DNA damage. Urine analysis, which is not generally considered to be directly applicable to genetic risk evaluation, was found to be positive.



Carcinogenicity: No carcinogenicity study is done with the current formulation. Available carcinogenicity data for the Naltrexone and PLG polymer is discussed later in this section.

Reproductive toxicology: The Revia NDA contains Segment I fertility and general reproductive toxicity study of naltrexone administered via gavage to Cr:Rats. The doses tested were 0, 20, 60, and 200 mg/kg/day. One female rat died (1/30) after 12 days of drug administration (high dose). The dam was described as thin in appearance. Necropsy findings indicated hemorrhage/brown color in the lungs, pitted surface in the

heart, and enlargements in thymus, mediastinal lymph nodes, and caecum. In addition a membrane-encased mass of soft, co animalsagulated white opaque material was found to fill the thoracic cavity and encase the lungs and heart and/or ungroomed coat, and vocalization was seen both in male and female. In males, dose related increase in excreted seminal plugs and hyperactivity/hypersensitivity, and in females chromorhinorrhea, were observed. Postmortem findings obtained at scheduled necropsy for both male and female rats indicated no drug related lesions. For F₀ generation female rats, the incidence of pseudopregnancy was significantly increased at high dose animals (7-20%) compared with the vehicle. The incidence for rats with any resorptions were 3 (27.3%), 3 (21.4%), 8 (66.7%), and 8 (80%) for vehicle, low, mid, high dose of naltrexone, respectively. This corresponds to average resorptions per litter of 0.3, 0.2, 1.2, and 2.6. All resorptions were early. No change in the average of corpora lutea, implantations litter sizes (live and dead), live fetuses, and the incidence of fetal gross external malformations was seen after naltrexone administration. Likewise, there were no alterations in fetal sex ratio or average fetal weights evident.

Natural delivery data showed one low dose dam and one mid dose dam (mated with vehicle control) each had no surviving litter beyond day 2 post parturition. This finding may have been either a drug-mediated effect on maternal behavior (poor maternal care, e.g. failing to remove placentas and umbilical cords from live born pups) or one secondary to pup mortality. Administration of naltrexone to dams mated with vehicle control males significantly increased the absolute incidence of stillborn pups. Although not significant, the average number of stillborn pups per litter and the incidence of delivering stillborn pups were increased in treated dams mated either with treated or vehicle control males. The incidence of dams delivering stillborn pups in vehicle control (both males and females untreated), and 0, low, mid and high dose naltrexone groups was 2 (14.3%), 1 (8.3%), 1 (7.7%), and 3 (27.3%), respectively with an average of 0.1, 0.2, 0.1 and 0.3 stillborn pups per litter. In these same respective groups, the incidence of stillbirth was 2 (1.4%), 2 (1.4%), 1 (0.6%), and 3 (2.0%). One (11.1%) of the vehicle control dams (mated with treated? males) and three (42.9%) of the treated dams (mated with vehicle control males) delivered stillborn pups; with averages 0.1 and 0.9 stillbirths per litter, respectively; the incidence of stillborn pups in these same respective groups was 1 (0.8%) and 6 (6.2%). Necropsy of F₁ generation rats showed no abnormalities in pups that either died during the first 21 days postpartum or on scheduled sacrifice at day 21 postpartum. Possible drug related behavioral signs were seen in F₁ generation pups, such as weak appearance, coldness to touch, and absence of nursing.

At weaning, average body weights of the pups born to high dose dams were significantly lower than the vehicle control rats; however, during the post weaning to cohabitation period average body weights were similar for all groups. A significant increase in average body weights of F₁ generation male rats was observed at scheduled sacrifice. No changes in average body weights of F₁ generation female rats were found throughout the gestation period. No drug related postmortem findings were disclosed for F₁ generation males or females either at scheduled sacrifice or at caesarean sectioning. The reproductive capacity of F₁ generation rats (including mating, fertility, caesarean

observations [both parenteral (F₁) and litter (F₂)] was not affected by naltrexone administration to the F₁ generation.

Teratology studies in rats and rabbits administered naltrexone (up to 200 mg/kg, oral) produced essentially no teratogenicity in F₁ generation rats and rabbits. In the rat study, naltrexone administration produced the expected behavioral signs and caused a transient, reversible dose related inhibition of maternal body weight gains. At the doses employed, naltrexone neither affected pregnancy rate nor any caesarean sectioning observations (both maternal and litter parameters). Fetal variations including gross external, soft tissue and skeletal variations and fetal ossification site averages were generally not affected. In the rabbit study, no teratogenic effects of naltrexone were demonstrated. However, dose related trends toward increased resorptions and average percentage of dead or resorbed implants/litter were observed. No other reproductive parameters, maternal or litter, were affected.

A perinatal and postnatal reproduction study in rats showed that naltrexone, up to 100 mg/kg, affected neither maternal (pregnancy and delivery) parameters in rats receiving naltrexone from day 13 of gestation to day 21 postpartum, nor litter parameters including pup mortality, litter size, live pups/litters pup survival, pup body weight, and pup sex ratio. At the doses employed, naltrexone produced the expected behavioral changes and caused a dose related inhibition of maternal body weight gain which persisted to day 1 of lactation.

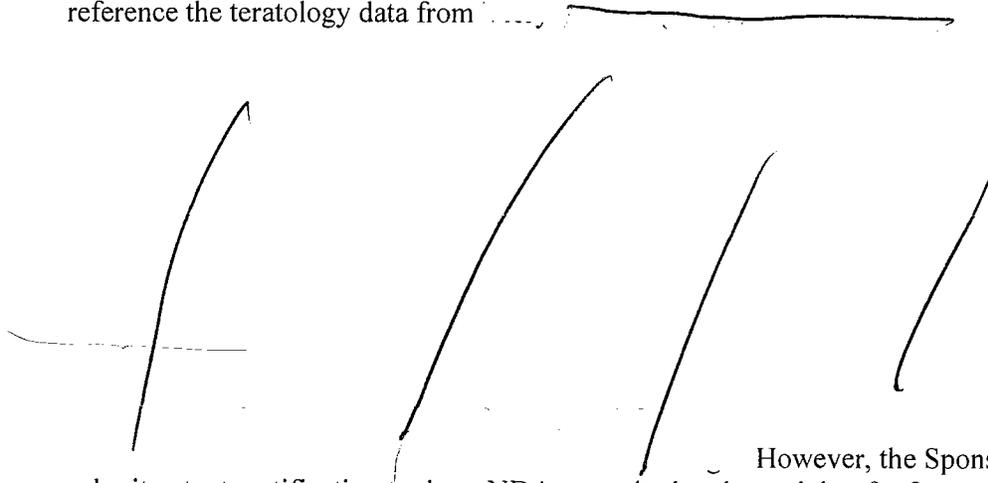
Segment III perinatal and postnatal reproductive toxicity study with naltrexone administered orally via gavage were done in (SD) female rats with 0, 10, 30, and 100 mg/kg/day. The animals were dosed from day 15 of gestation to day 21 post parturition. Necropsy was done at day 21 post parturition. Maternal observations include drug related slight to marked excess salivation seen in the high dosage group. Other minor clinical changes were noted, such as swollen axilla and/or chest. No drug related gross lesions were noted at necropsy. The lesions observed included moderate to marked dilatation of the pelvis of one or both kidneys in all dose group and a perforated esophagus and left axillary region engorged with a red-brown material in one high dose dam, and a tiny fluid filled area) in the left uterine born in another high dose dam. A significant, dose related inhibition of average maternal body weight gain was evident three days after drug administration (day 18 of gestation) and continued through day 20 of gestation. Maternal body weight change decreased with increased dose (averaged +34.6, +31.3*, +25.3** and +20.C** grams for days 15 to 18 of gestation and +59.6, +56.2, *52.4** and 45.4** grams for days 15 to 20 of gestation in the vehicle, low mid and high dose groups respectively (*=significant).

The dose-related decrease in average maternal body weight persisted to day 1 of lactation for dams administered nltrexone (283.7, 279.9, and 275.3 gms for vehicle, low, mid., and high dose respectively), thereafter dose-dependent increases in average maternal body weight gain from day 1 to 4 of lactation occurred (+6.5, +11.0, +12.0, and 13.2 gms, respectively). Neither effect, however, was significant. Subsequent average maternal

body weight gain during the remainder of the lactation period was similar for all dosage groups.

Administration of naltrexone did not affect the incidences of pregnancy, of dams surviving parturition, dams delivering pups, averages for implantation length of gestation, duration of parturition, or incidences of dams with stillborn or with one or more live pups, and of dams with no pups surviving to day 21 post parturition. Thin appearance, not nursing, cold to touch, decreased maternal care, weak appearance and lesion occurred in a non dose related fashion. No post mortem physical signs were observed in stillborn pups, or in pups which died during the 21-day post parturition period or pups that were sacrificed on day 21-post parturition were related to naltrexone administration to the F₀ generation dams.

The evaluation of the safety for PLG polymer in terms of potential reproductive-toxicity reference the teratology data from



However, the Sponsor did not submit patent certification to these NDAs, nor do they have right of reference to the data. Therefore, in the absence of the PLG data for Segment I and III reproductive toxicity study, it is recommended that the data for the above mentioned toxicity studies should be provided for labeling purposes. I do not believe that the lack of Segment I and III reproductive toxicology data should block approval of the NDA. It is my opinion that the studies may be completed as part of a phase 4 strategy.

Special toxicology: None

2.6.6.2 Single-dose toxicity

Study title: Oral Toxicity Screen with Glycolide Lactide Copolymer by Capsule Administration in Rabbits.

Key study Findings:

- Under the conditions of this study, the ALO for Glycolide Lactide copolymer was greater than 648 mg/kg of body weight.

Study Number:

Sponsor Reference No: AT-09-01

Conducting Laboratory:

The purpose of this test was to estimate the potential oral toxicity of glycolide/Lactide Copolymer by determining if the 'Approximate Lethal Dose' (ALD) is greater or less than approximately 500 mg/kg body weight. The ALD was defined as the lowest dose administered which caused death either on the day of dosing or within 14 days post exposure. Approximately 0.55 to 0.59 grams of test material per gelatin capsule was used. Three female rabbits ranging in weight from 2220 to 2360 grams were each dosed with 1 to 3 capsules. The dosing day was test day one; postexposure day 14 was test day 15. Rabbits were weighed on the day of dosing and during the recovery period of 14 days (excluding postexposure days 11 and 12). Rabbits were observed for clinical signs of toxicity and mortality on the day of dosing and twice daily on most days throughout the recovery period. The feces were also observed routinely, for any evidence of elimination of the test material through the gastrointestinal tract.

Due to an apparent obstruction of the trachea during dosing attempts, one rabbit was killed in extremis. In this study two rabbits were treated with approximately 500 or 648 mg/kg of the test material and observed for 14 days. The test rabbits were dosed with 2 and approximately 2 2/3 capsules, respectively. Both treated rabbits showed evidence of anorexia and one rabbit lost approximately 1% of its initial body weight one day after dosing. These responses were slight and were probably due to irritation off the throat incurred during balling gun dosing or were generalized reactions to stress. They are not considered to be toxicologically significant. Generally, rabbits continued to gain body weight throughout the recovery period. They showed no adverse clinical signs of toxicity and there was no mortality. On test day 6 the feces of one rabbit was white-coated and contained crystal appearing specks. From this observation, a small amount of test material was thought to be eliminated through the gastrointestinal tract

2.6.6.3 Repeat-dose toxicity

Study title: 1-Month Toxicokinetic Study of Medisorb® Naltrexone in Rhesus Monkeys with a 1-Month Recovery.

Key study findings:

- Under the conditions of this study, a single subcutaneous administration at doses up to 200 mg/kg and intramuscular administration at a dose of 50 mg/kg Medisorb® Naltrexone to male rhesus monkeys was well-tolerated.

- Subcutaneous and intramuscular administration of Medisorb® Naltrexone provided an initial release phase (during the first week) followed by a sustained-release phase. Dose proportional increases in C_{max} and AUC were observed after subcutaneous administration of Medisorb® Naltrexone. Compared to the subcutaneous dose, intramuscular administration of Medisorb® Naltrexone resulted in a similar plasma profile, although the initial naltrexone levels were slightly higher.
- Microscopically, at the terminal sacrifice, the subcutaneous injection sites with the test material (Medisorb® Naltrexone with 20, 50, or 200 mg naltrexone/kg) and the injection sites with the control material (Medisorb® Naltrexone Placebo microspheres) were essentially similar, except for the inflammatory response and fragmentation of the microspheres. Compared to the placebo sites, the test material sites had an increased incidence and severity of inflammatory response and more fragmentation of the microspheres. All subcutaneous injection sites (control and test material) had a minimal to moderately severe fibrous response enveloping the material. A granulomatous response (macrophages and foreign body multinucleated giant cells) was also present in all groups and varied in severity from minimal to moderately severe.
- The intramuscular sites (Medisorb® Naltrexone with 50 mg naltrexone/kg) had an inflammatory response of similar incidence and severity to that noted in the subcutaneous test material injection sites. The intramuscular injection sites also had minimal to slight lymphoplasmacytic inflammatory response and microsphere fragmentation. All of the animals treated by intramuscular injection had minimal skeletal muscle regeneration in one or more of the dose sites. Two of the intramuscular injection sites exhibited a minimal fibrous response enveloping the injected material where the material was between muscle bundles. All injection sites had residual material at the time of evaluation.

Study no.:

6403-117

Sponsor Reference No: AT-21-02

Volume # and page #: 1, 1-489**Conducting laboratory and location:****Date of study initiation:** March 23, 2000**GLP compliance:** Yes**QA report:** Yes**Drug, lot #, and % purity:** 190-0292B, Vehicle: placebo Medisorb microspheres, lot no: 191-2738B**Methods**

Doses: 20, 50, 200 mg/kg SC and 50 mg/kg IM

Species/strain: Monkeys *Macca mulatta*

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume, and infusion rate: SC; Medisorb-Naltrexone
Satellite groups used for toxicokinetics or recovery: 2/sex/group at high dose
were used for recovery

Age: 1.5-3 years old

Weight: 2.4-4.1 kg

Sampling times: Blood (approximately 1 mL) was collected predose;
approximately 2, 8, 24, 36, and 48 hours after each animal's last injection on Day
1; once on Days 5, 8, 11, 15, 18, 22, 26, and 30; and once on Days 34, 38, 45, 53,
and 60 for recovery animals.

Unique study design or methodology (if any): Doses were selected, by the
Sponsor, based on naltrexone exposure level in monkeys compared to humans.
The highest dose (200 mg/kg naltrexone) in monkeys was estimated to provide
naltrexone level of approximately 25X human AUC. The high dose was 4- and
10-fold higher than the selected mid- and low-dose levels (50 and 20 mg/kg
naltrexone, respectively). On Day 1, Medisorb® Naltrexone and Medisorb®
Naltrexone placebo microspheres were reconstituted with 1.5 ml
Diluent (approximate total suspension volume of 1.75 mL) and were administered
within approximately 5 minutes of reconstitution. Approximate suspension
concentrations were 140 mg/mL Medisorb® Naltrexone microspheres (50 mg/mL
naltrexone) and 145 mg/mL placebo microspheres. Both subcutaneous and
intramuscular injections were used because the intended route of administration in
humans was not determined at the time of the study and would either be
subcutaneous or intramuscular. Each animal was anesthetized with ketamine
(approximately 10 to 15 mg/kg) for dose administration.

Experimental Design:

Group	Dose Route ^a	No. of Animals	Nominal Naltrexone Dose Level ^b (mg/kg)	Dose Volume ^b (mL/kg)
1 (Control) ^c	SC	3	0	3.77
2 (Low)	SC	4	20	0.38
3 (Mid)	SC	4	50	0.94
4 (High) ^d	SC	6	200	3.77
5 (Mid)	IM	4	50	0.94

- a Animals in Groups 1 through 4 were dosed via subcutaneous injection (SC) and animals in Group 5 were dosed via intramuscular injection (IM).
- b Medisorb® Naltrexone contained — of active drug in microspheres. Each vial of Medisorb® Naltrexone was suspended at approximately 50 mg naltrexone/mL. Multiple injections were administered as required based on the dose and body weight (up to 1.5 mL/site).
- c The control animals received the Medisorb® Naltrexone placebo microspheres (approximately 145 mg/mL) at a microsphere dose equivalent to the high dose.
- d Two animals in the high-dose group were placed on recovery for an additional 30 days.

Observations and times and Results:

Mortality: The animals were observed twice daily (a.m. and p.m.) for mortality and morbidity. All animals survived to the end of the study.

Clinical signs: Each animal was observed daily for clinical signs and abnormal findings were recorded. Once weekly, detailed observations were made for each animal; abnormal findings or an indication of normal was recorded. There were no test material-related clinical observations during either the treatment (Days 1 through 31) or recovery periods (Days 32 through 61). Vomiting, abnormal feces, or low food consumption were observed for some treated animals; however, these findings were noted infrequently, were not observed in a dose-dependent manner, and were, therefore, not considered to be related to administration of the test material.

Injection Site Observations. The injection sites of each animal were examined for possible irritation two times/week (on nonconsecutive days). Any remarkable observations were identified and the dose deposition sites were measured (length x width). Subcutaneous and intramuscular dosing of Medisorb® Naltrexone and subcutaneous administration of Medisorb® Naltrexone Placebo microspheres were well-tolerated in monkeys. Intramuscular injection sites were not visible. Subcutaneous injection sites showed a localized firm enlargement attributed to the presence of test material depot (0.4 mL to 1.6 mL dose volume). When measured, these enlargements varied in size among sites. The sizes of injection sites on Day 1 (length x width) ranged from 28 x 27 mm to 15 x 14 mm. Generally, these sites were largest immediately following dosing on Day 1 and gradually became smaller during the 1-month period; the greatest decrease in size occurred during Days 1 through 4. Clinically, Medisorb® Naltrexone sites were similar to the placebo microsphere sites. However, placebo microsphere sites were slightly smaller in size on Day 29 compared to Medisorb® Naltrexone sites. The subcutaneous injection site of one animal given 20 mg/kg and three of three subcutaneous injection sites of one animal given 50 mg/kg were softer and swollen in appearance compared to other sites during the third and fourth weeks (to Day 29). One subcutaneous injection site of one animal given 200 mg naltrexone/kg was softer on Day 25 compared with the other sites. Microscopically, the injection sites that had clinical softness and swelling were not remarkably different from the other dose sites. There was little change in the size of the Medisorb® Naltrexone subcutaneous injection sites during recovery compared with the Day 29 observations. The injection site sizes at the end of recovery ranged from 18 x 14 mm to 15 x 11 mm.

Body weights: Individual body weight data were recorded before initiation of treatment, on the first day of treatment, and weekly thereafter. There were no test material-related effects on body weights.

Food consumption: Food consumption was assessed qualitatively, once daily. There were no test material-related effects on food consumption.

Ophthalmoscopy: Not done.

EKG: Not done.

Hematology: Blood samples for hematology, and coagulation (approximately 2, 1.8, and 1.5 mL, respectively) were collected twice before initiation of treatment and on Days 4, 31, and 61. The following hematological parameters were analyzed:

Red blood cell (erythrocyte) count; White blood cell (leukocyte) count; Hemoglobin; Differential blood cell count; Hematocrit; Segmented neutrophil count; Mean corpuscular volume; lymphocyte count; Mean corpuscular hemoglobin; Monocyte count; Mean corpuscular hemoglobin concentration; Eosinophil count; Basophil count; Platelet count; Blood cell morphology; Prothrombin time; Activated partial thromboplastin time. No changes in the above-mentioned parameters were noted.

Clinical chemistry: Blood samples for clinical chemistry tests (approximately 2, 1.8, and 1.5 mL, respectively) were collected twice before initiation of treatment and on Days 4, 31, and 61. The following clinical chemistry parameters were analyzed:

Glucose; Alanine aminotransferase; Urea nitrogen; Alkaline phosphatase; Creatinine; Gamma glutamyltransferase; Urea nitrogen/creatinine ratio; Aspartate aminotransferase; Total protein; Lactate dehydrogenase; Albumin; Creatine kinase; Globulin; Calcium Albumin/globulin ratio; Inorganic phosphorus; Total bilirubin; Sodium; Cholesterol; Potassium; Triglycerides; Chloride. No changes in the above-mentioned parameters were noted.

Urinalysis: Urine was collected for urinalysis tests once before initiation of treatment and on Days 31 and 61. The following parameters in urinalysis were analyzed: Urine volume; Bilirubin; Specific gravity; Blood pH; Urobilinogen; Protein; Microscopic examination of sediment; Glucose; appearance; Ketones. No changes were noted.

Gross pathology: Necropsy. On Day 31, each animal was bled for clinical pathology tests and three control and four treated animals/group were anesthetized with sodium pentobarbital, weighed, exsanguinated, and necropsied (terminal sacrifice). On Day 61, the two remaining animals given 200 mg/kg were bled for clinical pathology tests, anesthetized with sodium pentobarbital, weighed, exsanguinated, and necropsied (recovery sacrifice). The necropsy included an examination of the injection sites; a macroscopic examination of the external surface of the body; external body orifices; the thoracic, abdominal, and cranial cavities; organs; and tissues. Macroscopic injection sites were photographed. The majority of the injection sites in all groups exhibited tan and firm areas. There were no clear or consistent differences in the macroscopic observations between the control and test material-treated sites. No test material-related systemic effects were found in the macroscopic and microscopic evaluations, including the liver.

Organ weights: At each scheduled sacrifice, the following organs were weighed; paired organs were weighed together: adrenal (2), pituitary (postfixation), brain; spleen; heart; testis (2) with epididymis; kidney (2); thymus; liver.

Organ-to-body weight percentages and organ-to-brain weight ratios were calculated. There were no test material-related effects on terminal body weights or organ weights.

Histopathology: Adequate Battery: Yes; peer review: Yes.

The following tissues were preserved in 10% neutral-buffered formalin: adrenal (2), kidney (2), all injection dose sites lesions, aorta, liver, brain, lung with mainstem bronchi, cecum, lymph nodes, colon, duodenum, mammary gland, eyes with optic nerve [preserved in Davidson's fixative], pituitary, pancreas, gallbladder, prostate, heart, rectum, sciatic nerve, ileum, seminal vesicle (2), jejunum, skeletal muscle (thigh). Microscopically, at the terminal sacrifice, subcutaneous injection sites with the test material (Medisorb® Naltrexone with 20, 50, or 200 mg naltrexone/kg) and the subcutaneous injection sites with the control material (Medisorb® Naltrexone placebo microspheres) were essentially similar, except for the inflammatory response and fragmentation of the microspheres. Compared to the placebo control sites, the test material sites had an increased incidence and severity of inflammatory response and more fragmentation of the microspheres. There was a dose-dependent increase in lymphoplasmacytic inflammatory response, usually minimal to moderate but moderately severe in one of three sites for a Group 3 animal. All subcutaneous injection sites (control and test material) had a minimal to moderately severe fibrous response enveloping the material. A granulomatous response (macrophages and foreign body multinucleated giant cells) was also present in all groups. All of the injection sites had residual material at the time of evaluation. Microscopically, the subcutaneous injection sites that had clinical softness and swelling were not remarkably different from the other dose sites. The intramuscular injection sites (Medisorb® Naltrexone microspheres with 50 mg/kg naltrexone) had an inflammatory response of similar incidence and severity to that noted in the subcutaneous test material injection sites. The intramuscular injection sites also had minimal to slight lymphoplasmacytic inflammatory response and microsphere fragmentation. All of the animals treated by intramuscular injection had minimal skeletal muscle regeneration in one or more of the dose sites. Two of the intramuscular injection sites exhibited a minimal fibrous response enveloping the injected material where the material was between muscle bundles.

For recovery evaluation, two animals received the subcutaneous test material at a dose of 200 mg naltrexone/kg and were on study an additional 30 days. Macroscopically and microscopically, the injection sites appeared similar to the subcutaneous injection sites at the terminal sacrifice. The subcutaneous injection sites (test material) at the recovery sacrifice had a minimal to moderate fibrous response enveloping the material. A granulomatous response (macrophages and foreign body multinucleated giant cells) was also present in all injection sites. The granulomatous inflammation varied, between injection sites and the two animals, from minimal to moderately severe. The multinucleated giant cell response was variable but was still prominent in the recovery test material-treated animals. In the recovery animals, acute inflammatory cell response was minimal and present in only one injection site. The recovery test material sites also had a minimal to moderate lymphoplasmacytic inflammatory response. No test material-related systemic effects were found in the macroscopic evaluations or in the microscopic evaluation of the liver.

Summary of Microscopic Injection Site Findings
Incidence (% of Injection Sites affected)

Group	1	2	3	4	4 ^a	5
Number of Animals	3	4	4	4	2	4
Total Number of Injection Sites	26	4	10	33	19	9 ^b
Lesions						
Elevation, skin surface	2 (8)	0 (0)	4 (40)	24 (73)	11 (58)	0 (0)
Fibrous response around foreign material	26 (100)	4 (100)	10 (100)	33 (100)	19 (100)	2 (22)
Foreign material, fragmented spherical Structures	6 (23)	4 (100)	9 (90)	29 (88)	18 (95)	7 (78)
Foreign material, refractile spherical Structures	26 (100)	4 (100)	10 (100)	33 (100)	18 (95)	9 (100)
Hemorrhage	5 (19)	2 (50)	3 (30)	12 (36)	0 (0)	1 (11)
Inflammation, acute	3 (12)	3 (75)	7 (70)	13 (39)	1 (5)	5 (56)
Inflammation, granulomatous with Multinucleated giant cells	26 (100)	4 (100)	10 (100)	33 (100)	19 (100)	9 (100)
Inflammation, lymphoplasmacytic	4 (15)	3 (75)	10 (100)	31 (94)	16 (84)	9 (100)
Thrombus	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)
Degeneration of skeletal muscle	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)
Inflammation, subacute, dermis	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)
Regeneration of skeletal muscle	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (89)

a Recovery animals

b Injection site B was not examined microscopically for Animal No. I06950.

Toxicokinetics: Subcutaneous and intramuscular administration of Medisorb® Naltrexone provided an initial release phase (during the first week) followed by a sustained-release phase. Dose proportional increases in C_{max} and AUC were observed after subcutaneous administration of Medisorb® Naltrexone. The mean ± SD C_{max} values were 32 ± 8.4, 64 ± 6.4, and 265 ± 25 ng/mL for the Medisorb® Naltrexone at doses of 20, 50 and 200 mg naltrexone/kg, respectively. The corresponding subcutaneous AUC(1-month) levels were 187 ± 46, 449 ± 155, and 2115 ± 645 ng*d/mL, respectively. Compared to the subcutaneous dose, intramuscular administration of Medisorb® Naltrexone at 50 mg naltrexone/kg resulted in a similar plasma profile, although the initial naltrexone levels were generally somewhat higher during the first week. The intramuscular C_{max} and AUC values were 106 ± 30.0 ng/mL and 609 ± 21 ng*d/mL. In this study, intramuscular administration of Medisorb® Naltrexone provided a less variable PK profile compared to that of the subcutaneous route at the 50 mg naltrexone/kg dose. The plasma profiles of 6β-naltrexol for all groups tested were parallel to the naltrexone profiles but the levels were approximately 10 to 20 times lower than the mean naltrexone values. At the last sample point of 1-month, naltrexone concentrations for Medisorb® Naltrexone subcutaneous groups were dose-related with values of 2.86 ± 1.06, 4.06 ± 1.04, and 21.3 ± 5.03 ng/mL for the 20, 50, and 200 mg naltrexone/kg doses, respectively. At the last 1-month sample point for Medisorb® Naltrexone intramuscular group (50 mg naltrexone/kg), naltrexone concentration was 7.60 ± 2.66 ng/mL which was somewhat greater than the corresponding subcutaneous concentration. In the recovery animals that received Medisorb® Naltrexone at 200 mg naltrexone/kg, low circulating naltrexone levels were still present two months post-dose.

Comparison of Mean Dose Normalized Naltrexone Pharmacokinetic Parameters Among Subcutaneous Dose Levels of Medisorb Naltrexone in Monkeys

Dose, mg/kg	20 mg/kg, SC (N=4)		50 mg/kg, SC(N=4)		200 mg/kg, SC (N=6)	
	Mean	SD	Mean	SD	Mean	SD
C _{max}	31.8	8.42	64.3	6.38	265	24.5
T _{max} , Days	3.57	6.97	2.69	4.88	2.40	5.68
AUC _{0-1d} , ng · d/mL	19.2	3.28	44.4	2.44	179	18.7
AUC _{0-7d} , ng · d/mL	64.3	9.35	143	16.1	708	159
AUC _{0-29d} , ng · d/mL	187	45.7	449	155	2115	654

Comparison of Mean Dose Normalized 6β-Naltrexol Pharmacokinetic Parameters Among Subcutaneous Dose Levels of Medisorb Naltrexone in Monkeys

Dose, mg/kg	20 mg/kg, SC (N=4)		50 mg/kg, SC(N=4)		200 mg/kg, SC (N=6)	
	Mean	SD	Mean	SD	Mean	SD
C _{max}	1.62	0.79	4.86	1.14	12.3	2.01
T _{max} , Days	4.98	6.23	4.17	6.62	1.56	1.88
AUC _{0-1d} , ng · d/mL	1.07	0.57	3.37	0.90	8.57	1.70
AUC _{0-7d} , ng · d/mL	6.37	3.57	19.3	5.66	56.3	6.60
AUC _{0-29d} , ng · d/mL	11.1	5.55	40.6	12.5	127	22.7

Study title: A 3-Month Repeated-Dose Toxicokinetic Study of Medisorb® Naltrexone Administered by Subcutaneous Injection to Rhesus Monkeys, with a 3-Month Recovery Period.

Key study findings:

- No systemic toxicity reported following repeated subcutaneous treatment with Medisorb® Naltrexone in monkeys after 25, 50 and 75 mg/kg Medisorb Naltrexone administration.
- Toxicological findings were limited to the local injection sites. Based on clinical and gross evaluations, the residual microspheres gradually degraded over time.
- Microscopically, the injection site reactions consisted of a foreign body response with granulomatous inflammation and fibrous encapsulation. The severity of these responses declined with increasing time from dose administration. Although injection site reactions had not totally recovered after six months, continuing resolution was evident.
- The occurrence of anti-Naltrexone antibodies in 3 of 22 Medisorb® Naltrexone treated animals (13.6%) was not considered to have adversely impacted the study

since toxicokinetic data did not indicate that antibody interfered with drug exposure.

- Dose-related increases were observed in AUC and C_{max} for naltrexone and the metabolite, 6 β -naltrexol. C_{max} and AUC values tended to be larger in male monkeys compared to female monkeys.
- Repeat dosing resulted in a slight accumulation of naltrexone. Predose concentrations were measurable in most animals prior to receiving the second dose on Day 28, however, concentrations collected prior to the third dose and 28 days after the third dose did not increase further. The shape of the plasma concentration-time profiles of 6 β -naltrexol for all groups were similar to the naltrexone profiles but the mean concentrations of 6 β -naltrexol were about 10-times lower than the mean naltrexone values.

Study no.:

— 0944-54

Sponsor Reference No: AT-21-03

Volume # and page #: 1, 1-646

Conducting laboratory and location: _____

Date of study initiation: June 8, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: Lot # 190-1380A, 190-1300A, Placebo Lot # 191-1450A,
— Diluents Lot # Fo3A8; Placebo Microsphere Lot #191-1450A

Methods

Doses: 20, 50, 75 mg/kg

Species/strain: *Macaca mullta*

Number/sex/group or time point (main study):3/sex/group

Route, formulation, volume, and infusion rate: Subcutaneous, volume differs with different dose.

Satellite groups used for toxicokinetics or recovery: 2/sex/group high dose animal were kept for recovery.

Age: Male: 4-7.4 years; Females: 2.6-4.2 years

Weight: Male: 5-7.4 kg; Females: 2.8-4.1 kg

Sampling times: Approximately 1 mL of blood was collected in potassium EDTA-containing tubes at the following timepoints:

Day 1: Predose, 2 and 8 hours postdose

Day 2: 24 and 36 hours postdose

Days 3 (48 hour postdose), 5, 8, 12, 15, 19, 22 and 26

Day 29: Predose, 2 and 8 hours postdose

Day 30: 24 and 36 hours postdose

Days 31: (48 hour postdose), 33, 36, 40, 43, 47, 50 and 54

Day 57: Predose, 2 and 8 hours postdose

Day 58: 24 and 36 hours postdose

Days 59 (48 hour postdose), 61, 64, 68, 71, 75, 78, 82, 85 and 90
 Days 97, 104, 111, 118, 125, 132, 139, 146, 153, 160, 167, 174 and 180 (for recovery animals)

Unique study design or methodology (if any): Each animal received a dose of the test or control article once every 28 days (on Days 1, 29, and 57) via subcutaneous injection in the dorsal region. The animals were evaluated for changes in clinical signs, body weight, clinical pathology indices, injection site dimensions and sperm motility and morphology. Blood samples were collected for toxicokinetic analysis and serology at various timepoints throughout the study. Twenty-two animals (3/sex/group for Groups 2-4 and 2/sex/group for Group 1) were euthanized on Day 90 (except for Group 2 female R13834F and Group 3 male R13820M that were necropsied on Day 93 following a hypersensitivity skin test, see Section V.D.5). The remaining 6 animals (2/sex from Group 4 and 1/sex from Group 1) were maintained over an additional 3-month treatment-free period and terminated on Day 180. A full necropsy was conducted on all animals, and tissues were collected, preserved, processed, and examined by light microscopy. For the dosing procedures, the animals were lightly sedated with ketamine (to effect) by intramuscular injection. Once sedated, the dose site (back) was shaved and cleaned with alcohol prior to dosing. Each injection was at a different site that was uniquely identified throughout the study by using an indelible ink marker. The injection sites were re-marked during the study, as needed. On the days of dosing, the test and control articles were reconstituted with a 1.3 mL of diluent to yield proper concentrations. The total dose volume for each animal was based on the most recent body weight measurement. Based on the limitations of volume for a single injection, multiple injections, given in close proximity to each other, were required to achieve the target dose levels. Total dose volume for each animal was divided so that each injection did not exceed 1.5 mL. The maximum number of injections per dose cycle for each individual animal was not greater than five. Disposable sterile syringes were used for each injection. On the days of dosing, food was withheld from the animals prior to dosing. The food was provided once the animals had recovered from sedation.

Experimental Design:

Group No.	Number of Males/Females	Nominal Naltrexone Dose Level (mg/kg)*	Dose Volume (mL/kg)	Nominal Naltrexone Conc. (mg/mL)	Number Sacrificed on:	
					Day 90	Day 180
1	3/3	0 (control)**	0.86	--	2/2	1/1
2	3/3	20	0.23	87	3/3***	--
3	3/3	50	0.57	87	3/3***	--
4	5/5	75	0.86	87	3/3	2/2

* Medisorb® Naltrexone contained a nominal naltrexone load of 35%

** Group 1 received Medisorb® Naltrexone Placebo Microspheres (without active drug) at a microsphere dose equivalent to Medisorb® Naltrexone high dose (nominal microsphere concentration of 250 mg/mL and microsphere dose of 215 mg/kg).

***One Group 2 female and one Group 3 male were euthanized for necropsy on Day 93 to allow for skin (hypersensitivity) testing.

Observations, times and Results:

Mortality: Animals were observed within their cages at least twice daily throughout the study. Recording of cageside observations began 5 days prior to the first dose and continued until termination (Day 90/93 or 180). Each animal was observed once each morning and afternoon throughout the study for changes in general appearance and behavior. No mortality occurred in this experiment.

Clinical signs: Recording of cageside observations began 5 days prior to the first dose and continued until termination (Day 90/93 or 180) for observing clinical signs. Each animal was observed once each morning and afternoon throughout the study for changes in general appearance and behavior.

Several animals exhibited sporadic watery-liquid stool that was not dose-related in severity. The time of onset of the episodes was highly variable and not related to the times of dosing. An animal from Group 4 (R13827F) had a rectal prolapse on Day 64 that required a resection of the rectum; this animal recovered soon after the resection procedure. Rectal prolapse is occasionally seen in non-human primates under stressful conditions and was not attributed to the test article.

Body weights: Body weights were measured during prestudy, on Day 1, and weekly thereafter. No changes were found.

Food Consumption: Food consumption was qualitatively assessed daily for each animal (as part of the cageside observations), beginning 5 days prior to the first dose. The number of biscuits remaining from the previous feeding was examined, and a notation was made when less than approximately half of the rations were consumed. No test article related changes were observed.

Injection Site Observations and Measurements: The animals were removed from their cages, and the injection sites were examined immediately following dosing and twice weekly on non-consecutive days, thereafter. Any remarkable observations pertaining to the injection site were identified. The enlargements at the subcutaneous injection sites (due to the presence of the test or control material) were measured (length and width), as appropriate.

Raised areas of skin, due to the physical presence of the depot material (Medisorb® Naltrexone or Medisorb® Naltrexone Placebo Microspheres), were apparent at all injection sites following subcutaneous administration. The size of the injection site enlargement was variable among animals within each group and among the multiple injection sites for individual animals. In general, the size of the raised area was related to the absolute injection volume (ranging from 0.5 to 1.5 mL per site). The majority of sites were still visibly detectable at the Day 89 observation. Some sites, notably the Day 29 and 57 dosed sites, were still visibly detectable in the Group 4 recovery animals at the Day 177 observation. The majority of sites from all three dosing days were still detectable through the Day 177 observation in the Group 1 recovery animals. In most

animals, there were no further clinical findings involving injection sites except for gradual reduction in size during the study and those noted below.

Changes in skin coloration at the injection sites were limited to observations of sporadic redness in a few sites. Seven of 22 animals treated with Medisorb® Naltrexone had individual injection sites that were soft to the touch and/or that eventually ruptured to the surface. Further data analysis showed that injection site softness and rupture occurred sporadically among the 164 injection sites in Medisorb® Naltrexone-treated animals during the three cycles of treatment. The incidence of soft and/or ruptured injection sites for each of the three treatment cycles was 17-20%. Generally, in animals with multiple injection sites, all active sites were not involved. As a percentage of total sites per dose group, the incidence of rupturing and/or softening was similar among the test article-treated groups. In addition, injection site rupturing or softening appeared unrelated to the exact injection volume, since affected sites had been injected with volumes ranging from 0.7 to 1.5 mL.

The range of time following each dose when injection sites first became soft to the touch or ruptured was variable among animals (7 to 67 days post-dose). The absence of any histologic findings in ruptured sites that would account for this may be related to the latency between rupturing of the sites and the necropsy of the animals, as well as the fact that the test material was apparently lost from these sites.

It was concluded that Medisorb® Naltrexone subcutaneous injection sites in monkeys were more susceptible to swell/rupture, although mere presence of the formulation was not considered sufficient to produce the effect. A precipitating factor was not identified but it is possible that minor skin trauma and subsequent local infection could have played a role. Minor skin trauma is a relatively common occurrence in monkeys under laboratory conditions.

Incidence of Injection Site Rupture or Softening as the Endpoint

Dose Day	Group No.	No. of Animals	Total No. of Sites Dosed	No. of Sites Ruptured / Soft	Number of Ruptured (R) or Soft (S) Injection Sites per Animal - First Day of Rupture or Occurrence of Soft Sites (that did not rupture)
Day 1 Sites	1	6	18	0 / 0	—
	2	6	7	0 / 1	R13834F (1S) - Day 47
	3	6	14	3 / 0	R13820M (3R) - Days 22-36
	4	10	33	2 / 4	R13812M (1R) - Day 8* R13810M (1R) - Day 29 R13826F (2S) - Day 54 R13840F (2S) - Day 82 only
Day 29 Sites	1	6	19	0 / 0	—
	2	6	7	1 / 0	R13834F (1R) - Day 43
	3	6	14	3 / 0	R13820M (3R) - Days 33-36
	4	10	33	2 / 3	R13826F (2R) - Days 54-64 R13801M (3S) - Days 57-61
Day 57 Sites	1	6	21	0 / 0	—
	2	6	7	1 / 0	R13834F (1R) - Day 68
	3	6	14	3 / 0	R13820M (3R) - Day 61
	4	10	35	3 / 4	R13826F (2R) - Day 64 R13801M (1R) - Day 64 R13801M (4S) - Day 68

* site was reddened on Day 8; day of rupture was not documented; scar noted on Day 78

Summary of Injection Site Rupture or Softening (as Total Sites Dosed Endpoint) vs. Total Sites Dosed

Group No.	Ruptured Sites / Total Sites Dosed	Percent (%) Ruptured Sites	Sites Soft Only / Total Sites Dosed	Percent (%) Soft Sites	Percent (%) Ruptured & Soft Sites
1	0/58	0%	0/58	0%	0%
2	2/21	10%	1/21	5%	14%
3	9/42	21%	0/42	0%	21%
4	7/101	7%	11/101	11%	18%

Summary of Injection Site Rupture or Softening (as Endpoint) vs. Total Sites Dosed with Medisorb® Naltrexone per Dosing Cycle

Dosing Day	Total Sites Dosed with Test Article	No. of Sites Ruptured or Soft	Percent (%) Ruptured & Soft Sites
1	54	10	19%
29	54	9	17%
57	56	11	20%

Diagnostic Skin Testing

Based on the above-described changes in the appearance of the injection sites, a diagnostic test was performed on two selected animals to investigate whether the changes may have been associated with an immunologic reaction to the test article or its constituents. Group 2 animal R13834F and Group 3 animal R13820M were selected for a hypersensitivity skin test. The animals chosen were among those in which injection sites at all three dosing intervals became soft to the touch and/or ruptured. The test consisted of administration of the primary components of Medisorb® Naltrexone in the abdominal region by intradermal injections (Diluent, and 10 mM naltrexone HCl), and by subcutaneous injection (Medisorb® Naltrexone Placebo Microspheres) at 0.1 mL dose volume per injection. An additional intradermal injection (0.1 mL) of control material (Saline; 0.9% NaCl, USP for injection) was included. These injection sites were observed over 72 hours. At 24 hours, both animals had a slightly reddened area at the site of the 10 mM naltrexone HCl injection, approximately the same size as it was at the time of the dose administration. The slight reddening remained during the observation period; however, the area became smaller and increasingly indistinct. Although some irritation was suggested by the reddening, the severity of the reaction was

judged to be insufficient to demonstrate the presence of either immediate or delayed hypersensitivity to naltrexorie. In addition, no changes were noted at any other test site to suggest hypersensitivity to other Medisorb® Naltrexone components.

Ophthalmoscopy: Not done

EKG: Not done

Hematology: Approximately 1 mL of blood was collected in EDTA-containing tubes. The whole blood samples were analyzed for the following parameters:

Hematology Table	
Red blood cell (RBC) counts	Mean cell hemoglobin (MCH)
White blood cells (WBCs)*	Mean corpuscular volume (MCV)
Hemoglobin concentration	Mean corpuscular hemoglobin concentration (MCHC)
Hematocrit	Platelet counts
Reticulocyte counts	Blood cell morphology**

*Total and differential: Included polysegmented neutrophils, band cells, lymphocytes, monocytes, eosinophils, and basophils

** The blood smears from all animals were examined at each timepoint (including prestudy).

No changes in the hematological parameters were noted.

Coagulation Parameters: Approximately 1.8 mL of blood was collected in tubes containing sodium citrate anticoagulant; the samples were centrifuged to obtain plasma, and the plasma was analyzed for the following parameters:

Activated partial thromboplastin time (APTT)
Prothrombin time (PT)

No changes were noted.

Clinical chemistry: Approximately 2 mL of blood was collected in a tube without anticoagulant. The sample was allowed to clot and then centrifuged to obtain serum. The serum was analyzed for the following parameters:

Serum Chemistry Table	
Sodium	Calcium
Potassium	Phosphorus
Chloride	Urea nitrogen (BUN)
Total bilirubin	Creatinine
Alkaline phosphatase (AP)	BUN/creatinine ratio
Lactate dehydrogenase (LDH)	Total protein
Aspartate aminotransferase (AST)	Albumin
Alanine aminotransferase (ALT)	Globulin
Gamma-glutamyltransferase (GGT)	Albumin/globulin ratio
Glucose	Cholesterol
Creatine Phosphokinase (CPK)	Triglycerides

No changes in the serum chemistry parameters were noted.

Urinalysis: Urine samples were obtained from cagepan collection prestudy and from the bladder at necropsy, and were analyzed for the following parameters:

Urinalysis Table	
Color/Character	Protein
pH	Glucose
Specific gravity	Ketones
Leukocyte esterase	Bilirubin
Nitrite	Occult blood
Urobilinogen	Microscopics

No changes were noted.

Toxicokinetics: For the primary toxicokinetic analysis of naltrexone and 6 β -naltrexol, plasma concentration versus time data for individual animals was evaluated to determine the maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under plasma concentration-time curve (AUC) from time zero to Day 28. After subcutaneous administration of Medisorb® Naltrexone, a dose-related increase was observed in C_{max} and AUC for both naltrexone and the metabolite, 6 β -naltrexol. C_{max} and AUC values tended to be larger in male monkeys compared to female monkeys, however, the small number of animals involved in the study prevented meaningful statistical comparisons. Repeat dosing resulted in a slight accumulation of naltrexone. Predose concentrations were measurable in most animals prior to receiving the second dose on Day 28, however, concentrations collected prior to the third dose and 28 days after the third dose did not increase further.

The shape of the plasma concentration-time profiles of 6 β -naltrexol for all groups were similar to the naltrexone profiles but the mean concentrations of 6 β -naltrexol were about 10-times lower than the mean naltrexone values. 6 β -naltrexol exposure increased with increasing dose. As observed with naltrexone, slight accumulation of 6 β -naltrexol was observed prior to the second dose, which did not increase further.

Samples for Antibody Analysis: Approximately 1 mL of blood was collected in tubes without anticoagulant prior to dosing on Days 1, 29 and 57 and on Days 90 (all animals) and 180 (recovery animals). The samples were allowed to clot and then centrifuged to obtain serum. The serum was kept frozen until further analysis.

All serum samples were tested by a direct ELISA for anti-naltrexone antibody. Any positive samples from Day 57 were also tested in a competition assay to evaluate antibody specificity.

The purpose of this phase of the study was to determine if naltrexone-specific antibodies were formed following the subcutaneous administrations of Medisorb® Naltrexone. Serum samples were tested by direct ELISA for the presence of anti-naltrexone antibody. A positive anti-naltrexone control serum was obtained through the immunization of rabbits _____ with a naltrexone-BSA conjugate (constructed at Alkermes, Inc., Cambridge, MA).

One control monkey reacted at a low level to the anti-naltrexone antibody assay prior to and during the study despite no exposure to naltrexone. Three of 22 animals (13.6%)

treated with Medisorb® Naltrexone exhibited detectable at low to increased levels of anti-naltrexone antibody. Two animals treated with Medisorb® Naltrexone had a low level of anti-naltrexone antibody response beginning at Day 29 or 57. One animal had a low level at Day 29 followed by an increased response at Days 57 and 90. Serum samples from the three positive Medisorb® Naltrexone treated animals on Day 57 were determined to be specific for naltrexone based on a separate competition assay. In the same assay, antibody from the positive control animals was determined to have a lower specificity.

Gross pathology: A complete gross necropsy was conducted on all animals sacrificed during the study. The necropsy included examination of:

- Carcass and muscular/skeletal system
- All external surfaces and orifices
- Cranial cavity and external surface of the brain
- Neck with associated organs and tissues
- Thoracic, abdominal and pelvic cavities with their associated organs and tissues

All abnormalities were described completely and recorded.

The treatment-related gross findings were limited to the subcutaneous injection sites. Nodules were observed in most of the control-article and test-article injection sites in animals sacrificed on Days 90 - 93 and on Day 180. These were attributed to the presence of residual microspheres in the control article and test article, and/or to the tissue response to these microspheres. These observations are further described below.

a) Animals Sacrificed on Days 90 - 93

On Days 90 - 93, raised, white-tan nodules were present in most injection sites. In both control-article and test-article injection sites, these nodules were due to the residual microspheres and to the foreign-body response to the microspheres that was observed histologically. In these animals, the size of the nodules tended to decrease with increasing time after injection in both control-article and test-article injection sites. For each set of dose sites from the same administration day, there were no discernible differences between the sizes of nodules in control-article and test-article injection sites. Red discoloration associated with some of the injection sites correlated with the hemorrhage observed microscopically. All other gross lesions in these animals were considered incidental and unrelated to the control- or test-articles.

b) Animals Sacrificed on Day 180

In animals sacrificed on Day 180, the size of injection site nodules at each dose administration time-point was generally decreased, as compared to those in animals sacrificed on Days 90 - 93. In the two Group 1 (control) animals sacrificed on Day 180, a nodule persisted at each injection site for each dose-administration time-point. In the four Group 4 (high-dose) animals sacrificed on Day 180, nodules were observed in one of twelve Day 1 injection sites, eight of 12 Day 29 injection sites and in all (13) Day 57 injection sites. The size of the nodules persisting in these injection sites was also less in Group 4 animals than in Group 1 animals. These observations suggest that on Day 180, absorption of the Medisorb Naltrexone in Group 4 animals was more complete than was

absorption of the Medisorb Naltrexone Placebo microspheres in Group 1 (control). This interpretation must, however, be made in the context of the number of animals sacrificed on Day 180. Red discoloration in some injection sites on Day 180, as on Days 90 - 93, correlated with microscopically observed hemorrhage.

Sperm Analysis: Sperm analysis was conducted for all males at the time of necropsy with the exception of animal R13820M (Group 3) that was sacrificed on Day 93 and the 3 males that underwent the 3-month treatment-free period (Day 180 necropsy). The vas deferens and epididymis from the right testicle were collected and transferred (after weighing of the epididymis) to a representative of _____ present at necropsy. The _____ technician isolated sperm from the vas deferens and recorded the sperm motion parameters onto an optical disk drive using a _____ Sperm Analyzer. The epididymal samples were kept frozen until further analysis. The previously recorded sperm motion images were evaluated and a percent motility determined for each animal. One epididymis per male animal was processed, and the total count of sperm per gram of epididymis was determined. Sperm morphology was evaluated for a minimum of 200 sperm cells per animal.

Mean percent motility, total sperm count and sperm morphology were not affected by treatment with Medisorb® Naltrexone at dosage levels of 20, 50, and 75 mg naltrexone/kg. No biologically meaningful differences were observed between the study groups for any of the parameters examined.

Organ Weights For all animals, the following organs were weighed before fixation. Paired organs (except the epididymides) were weighed together unless gross abnormalities were present, in which case they were weighed separately. The pituitary was weighed post fixation.

Organ Weight Table	
Adrenals	Brain
Epididymides (weighed separately)	Heart
Kidneys	Liver
Pituitary (post fixation)	Ovaries
Testes	Spleen
--	Thymus

No appreciable changes in organ weight occurred.

Histopathology: Adequate Battery: Yes; peer review: Yes

The following tissues and organs (or portions of) were collected from all animals sacrificed and preserved in neutral-buffered 10% formalin (except for the eyes, which were preserved in 3% glutaraldehyde solution for optimum fixation).

Tissue Collection Table	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymis (left)
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine

Tissue Collection Table	
Large Intestine	Adrenals
Cecum	Pituitary
Colon	Thyroid/Parathyroids*
Rectum	Skin/Musculoskeletal
Pancreas	Skin/Mammary Gland
Liver	Bone (femoral head)
Gallbladder	Bone (7th rib)
Respiratory	Skeletal Muscle (thigh)
Trachea	Nervous/Special Sense
Lung	Eyes with optic nerve
Lymphoid/Hematopoietic	Sciatic Nerve
Bone Marrow (sternum)	Brain
Thymus	Spinal Cord (thoracic)
Spleen	Other
Lymph Nodes	Animal Number Tattoo
Mandibular	Gross Lesions
Mesenteric	Injection Sites on back

* The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

A bone marrow smear was collected from the seventh rib of all animals and was preserved for possible examination in the event that treatment-related changes were observed in the sternal bone marrow section that required further evaluation. As treatment-related changes were not evident in the sternal bone marrow section and hematological profiles, examination of the marrow smear was not necessary. For all animals, the tissues listed above were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Only slides from all tissues of animals in Groups 1 and 4 (except recovery animals), and the injection sites and gross lesions from all other animals were examined by light microscopy.

Treatment-related histologic findings were limited to the injection sites in animals sacrificed on Days 90 - 93 and Day 180. As these findings in the test-article groups were generally similar to those in the control-article group, they were attributed to the microspheres rather than to the naltrexone. The maximum volume per injection site was 1.5 mL; consequently, most animals had multiple injection sites on each dose administration day. As the histologic findings in all injection sites on each dose administration day were generally consistent, this was considered to be representative of the response to the control and test article formulations on each dose administration day. Injection sites contained microscopic changes attributed to the control-article and test-article formulations and are discussed in detail below.

a) Animals Sacrificed on Day 90/93

Most of the histologic findings in injection sites from animals sacrificed on Days 90 - 93 were attributed to the residual microspheres present in both the control-article and the test-article formulations. None of the histologic changes in injection sites in these animals were considered related to naltrexone. The injection sites in Group 1 (control-article) animals contained large clear vacuoles that were consistent with microsphere deposition. The histologic response to this material was a foreign body response that consisted primarily of fibrous encapsulation and granulomatous inflammation. A capsule of dense fibrous connective tissue surrounded the injected material and the granulomatous inflammation extended from the periphery toward the center of the injection site. The components of granulomatous inflammation consisted of multinucleated giant cells and large macrophages, with fewer lymphocytes and eosinophils. Plasma cells and neutrophils were rare. Fibrosis and fibroplasia were also minor components of the granulomatous inflammation. The proportion of each of these components tended to vary between injection sites. In some control-article and test-article injection sites, there were central regions of eosinophilia without cellular infiltrates that were consistent with accumulation of proteinic fluid (seroma). Some of these regions contained spaces or potential spaces and they were more prevalent in the larger injection sites. The largest of these areas were in the injection sites found to be soft when palpated clinically. These areas were diminished but persistent in a few injection sites from animals sacrificed on Day 180. They were attributed to the volume of the depot formulations injected in the loose subcutaneous tissue of these animals and were not considered related to specific components of either the control-article or to the test-article formulations.

In Group 1 animals (control-article) sacrificed on Days 90 - 93, the incidence and severity of fibrous encapsulation and granulomatous inflammation were similar in the Day 1, Day 29 and Day 57 injection sites. The incidences of fibrous encapsulation and granulomatous inflammation, in both males and females, were generally similar in the control-article and test-article groups in sites injected on Days 29 and 57. In sites injected on Day 1, the severity of both of these changes tended to be slightly greater in test-article formulation groups than in control-article formulation groups. The degree of variation between individual animals in the severity of these findings was generally similar in sites from each dose administration day. Given the absence of a difference in the severity of the local response between the control-article and test-article formulations on Days 29 and 57, and the similar degrees of individual animal variation in this response at all timepoints, the slightly greater severity of this response in test-article formulation sites as compared to control-article formulation sites on Day 1-could not be attributed to the test-article formulation with confidence. Hemorrhage in injection sites was sporadic and tended to occur in the fibrous capsule. It was attributed to the injection procedure as this was observed in control-article and test-article injected groups.

One or more injection sites ruptured in several animals in Groups 2, 3 and 4 as early as Day 22 and as late as Day 68. This included all sites in animal 3820M (Group 3) and two of the three sites in animal R13834F (Group 2). Most of these injection sites lacked a

fibrous capsule, contained few or no microspheres, and had only minimal granulomatous inflammation. Most of these sites also contained dermal fibrosis consistent with scarring. These changes were considered to represent the resolution of the response to these ruptures, but did not suggest a cause for the rupture of these injection sites. In two animals (R13826F and R13801M), some injection sites were soft on palpation for variable periods between Days 54 and 89. In addition to fibrous encapsulation and granulomatous inflammation, these injection sites also contained granulation tissue, pseudocystic spaces, and proteinic material consistent with seroma formation. The softness palpated clinically in these sites was most likely due to the granulation tissue and seromas.

b) Animals Sacrificed on Day 180

In animals sacrificed on Day 180, fibrous encapsulation and granulomatous inflammation remained the primary responses to both the control-article and test article formulations. Differences between control-article and test-article groups in severity of these changes, at this time, were slight and could not be clearly attributed to sex or treatment. These responses were slightly diminished (less severe) in injection sites from each dose administration day in animals sacrificed on Day 180 than in the corresponding injection sites in animals sacrificed on Days 90-93. The other histologic changes present in tissues evaluated from these animals were consistent with spontaneous histologic changes commonly seen in naive rhesus monkeys and were not test article related.

Summary of Histopathologic Findings Days 90-93

DAYS ON TEST: 90-93		SEX: MALE			
INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS					
GROUP:		1M (1)	2M (2)	3M (3)	4M (4)
NUMBER OF ANIMALS:		2	3	3	3
Injection Site, Day 1	# EX	2	3	3	3
Encapsulation, Fibrous		2	3	2	3
Hemorrhage		0	1	3	2
Inflammation, Granulomatous		2	3	3	3
Pigment, Hemosiderin		0	1	0	0

Injection Site, Day 1	# EX	2	3	3	3
Fibrosis		0	0	1	0
Injection Site, Day 29	# EX	2	3	3	3
Encapsulation, Fibrous		2	3	2	3
Hemorrhage		1	2	3	2
Inflammation, Granulomatous		2	3	3	3
Fibrosis		0	0	1	0
Depositon, Protein		1	0	0	1
Injection Site, Day 57	# EX	2	3	3	3
Degeneration, Myofiber		0	1	0	0
Encapsulation, Fibrous		2	3	2	3
Foreign Body, Hair		0	0	1	0
Granulation Tissue		0	0	1	0
Hemorrhage		1	3	3	3
Inflammation, Granulomatous		2	3	3	3
Ulcer		0	0	1	0
Fibrosis		0	0	1	1
Depositoin, Protein		0	0	0	1
Inflammation, Mixed Cell		0	0	0	1
Jejunum	# EX	2	0	0	3
Kidney	# EX	2	0	1	3
Atrophy, Tubular Epithelium		0	0	1	0
Degeneration, Tubular Epithelium		0	0	0	1
Fibrosis, Interstitial		0	0	1	0
Glomerulosclerosis		0	0	0	1
Infiltrate, Mononuclear Cell		1	0	1	2
Fibrosis, Periglomerular		0	0	1	0
Dilatation, Renal Tubule		0	0	1	0
Liver	# EX	2	1	0	3
Granuloma, Mineralized		0	1	0	1
Infiltrate, Mononuclear Cell		2	0	0	2
Inflammation, Chronic, Periportal		0	1	0	0
Vacuolation, Cytoplasm, Hepatocyte		0	0	0	2

Lung	# EX	2	0	0	3
Fibrosis, Pleura		1	0	0	0
Infiltrate, Mononuclear Cell, Perivascular		0	0	0	1
Pneumoconiosis		2	0	0	3

FINDINGS ALL					
DAYS ON TEST: 90-93					
INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS				SEX: FEMALE	

Injection Site, Day 1	# EX	2	3	3	3
Encapsulation, Fibrous		2	3	3	3
Hemorrhage		0	2	1	2
Inflammation, Granulomatous		2	3	3	3
		#	#	#	#
Injection Site, Day 29	# EX	2	3	3	3
Encapsulation, Fibrous		2	2	3	2
Hemorrhage		0	0	1	3
Inflammation, Granulomatous		2	3	3	3
Injection Site, Day 57	# EX	2	3	3	3
Encapsulation, Fibrous		2	2	3	2
Granulation Tissue		0	0	1	0
Hemorrhage		0	2	2	3
Inflammation, Granulomatous		2	3	3	3
Depositoin, Protein		0	0	0	1
Jejunum	# EX	2	0	0	3
Kidney	# EX	2	0	0	3
Atrophy, Tubular Epithelium		0	0	0	1
Cast, Proteinic, Renal Tubule		0	0	0	1
Fibrosis, Interstitial		0	0	0	1
Infiltrate, Mononuclear Cell		1	0	0	2
Liver	# EX	2	0	0	3
Cyst		1	0	0	0
Infiltrate, Mononuclear Cell		1	0	0	3
Inflammation, Chronic, Periportal		2	0	0	0
Inflammation, Granulomatous, Necrotizing		2	0	0	0
Necrosis, Hepatocellular		1	0	0	0
Pigment, Hemosiderin		1	0	0	0
Lung	# EX	2	0	0	3
Infiltrate, Macrophage, Alveolus		1	0	0	0
Pneumoconiosis		2	0	0	3

(1) - 0 mg/kg Naltrexone Dose Volume 0.86 mL/kg
 (2) - 20 mg/kg Naltrexone; Dose volume 0.23 mL/kg

(3) - 50 mg/kg Naltrexone; Dose Volume 0.57 mL/kg
 (4) - 75 mg/kg Naltrexone; Dose Volume 0.86 mL/kg

Summary of Histologic Findings, Day 180

DAYS ON TEST: 180-180

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:	1M (1)	4M (2)
NUMBER OF ANIMALS:	1	2
Injection Site, Day 1	# EX 1	# 2
Encapsulation, Fibrous	1	1
Hemorrhage	1	2
Inflammation, Granulomatous	1	2
Fibrosis	1	2
Injection Site, Day 29	# EX 1	# 2
Encapsulation, Fibrous	1	2
Hemorrhage	0	1
Inflammation, Granulomatous	1	2
Fibrosis	1	1
Injection Site, Day 57	# EX 1	# 2
Encapsulation, Fibrous	1	2
Hemorrhage	1	2
Inflammation, Granulomatous	1	2
Fibrosis	1	2
Lung	# EX 1	# 0
Infiltrate, Macrophage, Alveolus	1	0
Pneumoconiosis	1	0
Skin	# EX 0	# 1
Hemorrhage, Subcutis	0	1
Stomach	# EX 1	# 0
Hemorrhage, Mucosa	1	0
Erosion, Mucosa	1	0

DAYS ON TEST: 180-180

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:	1F (1)	4F (2)
NUMBER OF ANIMALS:	1	2
Injection Site, Day 1	# EX 1	# 2
Encapsulation, Fibrous	1	2
Hemorrhage	0	1
Inflammation, Granulomatous	1	2
Pigment, Hemosiderin	0	1
Fibrosis	1	0
Infiltrate, Macrophage	0	1
Injection Site, Day 29	# EX 1	# 2
Encapsulation, Fibrous	1	2
Inflammation, Granulomatous	1	2
Fibrosis	1	1
Injection Site, Day 57	# EX 1	# 2
Encapsulation, Fibrous	1	2
Inflammation, Granulomatous	1	2
Fibrosis	1	2

Severity of Selected Findings, Day 90-93

DAYS ON TEST: 90-93		SEX: MALE			
GROUP:		1M (1)	2M (2)	3M (3)	4M (4)
NUMBER OF ANIMALS:		2	3	3	3
Injection Site, Day 1	# EX	2	3	3	3
Encapsulation, Fibrous					
minimal		2	0	0	0
mild		0	2	1	3
moderate		0	1	1	0
Hemorrhage					
minimal		0	1	3	2
Inflammation, Granulomatous					
minimal		1	1	1	0
mild		1	1	0	1
moderate		0	1	2	2
Injection Site, Day 29	# EX	2	3	3	3
Encapsulation, Fibrous					
minimal		1	0	0	0
mild		1	3	1	2
moderate		0	0	1	1
Hemorrhage					
minimal		1	2	3	1
mild		0	0	0	1
Inflammation, Granulomatous					
minimal		0	0	1	0
mild		1	3	1	2
moderate		1	0	1	1
Injection Site, Day 57	# EX	2	3	3	3
Encapsulation, Fibrous					
minimal		1	1	1	0
mild		1	2	1	3
Hemorrhage					
minimal		1	2	0	3
mild		0	1	3	0
Inflammation, Granulomatous					
minimal		0	0	0	1
mild		2	3	3	1
moderate		0	0	0	1

Severity of Selected Findings, Day 90-93

DAYS ON TEST: 90-93		SEX: FEMALE			
GROUP:		1F	2F	3F	4F
		(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:		2	3	3	3
	#	#	#	#	#
Injection Site, Day 1	# EX	2	3	3	3
Encapsulation, Fibrous					
minimal		1	1	1	1
mild		1	2	1	2
moderate		0	0	1	0
Hemorrhage					
minimal		0	2	1	2
Inflammation, Granulomatous					
minimal		2	0	0	0
mild		0	0	1	0
moderate		0	3	2	3
Injection Site, Day 29	# EX	2	3	3	3
Encapsulation, Fibrous					
minimal		0	0	1	0
mild		2	2	2	2
Hemorrhage					
minimal		0	0	1	3
Inflammation, Granulomatous					
minimal		0	2	0	1
mild		2	1	3	1
moderate		0	0	0	1
Injection Site, Day 57	# EX	2	3	3	3
Encapsulation, Fibrous					
minimal		0	0	1	1
mild		2	2	2	1
Hemorrhage					
minimal		0	2	1	3
mild		0	0	1	0
Inflammation, Granulomatous					
minimal		0	3	2	2
mild		2	0	1	1

Severity of Selected Findings, Day 180

DAYS ON TEST: 180-180		SEX: MALE	
GROUP:		1M	4M
		(1)	(2)
NUMBER OF ANIMALS:		1	2
		#	#
Injection Site, Day 1	# EX	1	2
Encapsulation, Fibrous			
mild		1	1
Hemorrhage			
minimal		1	2
Inflammation, Granulomatous			
minimal		0	1
mild		1	1
Injection Site, Day 29	# EX	1	2
Encapsulation, Fibrous			
mild		1	2
Hemorrhage			
minimal		0	1
Inflammation, Granulomatous			
minimal		0	1
mild		1	1
Injection Site, Day 57	# EX	1	2
Encapsulation, Fibrous			
mild		1	2
Hemorrhage			
minimal		1	2
Inflammation, Granulomatous			
minimal		1	0
mild		0	2

**APPEARS THIS WAY
ON ORIGINAL**

**APPEARS THIS WAY
ON ORIGINAL**

Severity of Selected Findings, Day 180

DAYS ON TEST: 180-180		SEX: FEMALE	
GROUP:		1F (1)	4F (2)
NUMBER OF ANIMALS:		1	2
		#	#
Injection Site, Day 1	# EX	1	2
Encapsulation, Fibrous			
minimal		0	2
mild		1	0
Hemorrhage			
minimal		0	1
Inflammation, Granulomatous			
minimal		0	2
mild		1	0
Injection Site, Day 29	# EX	1	2
Encapsulation, Fibrous			
minimal		0	1
mild		1	1
Inflammation, Granulomatous			
minimal		0	1
mild		1	1
Injection Site, Day 57	# EX	1	2
Encapsulation, Fibrous			
mild		1	2
Inflammation, Granulomatous			
mild		0	2
moderate		1	0

Study title: 90-Day, Three Repeat Dose Subcutaneous-Toxicity Study of Medisorb® Naltrexone (VIVITREX) in Rats (Draft Unaudited Report)

Key study findings:

- Three, once-per-month (Days 1, 30 and 58) injections were well tolerated for all dose groups (25, 100 mg/kg). One early death in the placebo group male animal (1/10) occurred at Day 11. The death was determined to be due to a urinary tract obstruction.
- Clinical observations of swelling at each of the injections sites over the course of the study were attributed to the microspheres in the formulation and not naltrexone. Major necropsy treatment-related findings were inflammation at the injection sites. This finding was observed in the Medisorb® Naltrexone and

Placebo groups in approximately equal incidence and severity. Thus, inflammation at these injection sites was attributed to the polymer microspheres and not to drug-specific toxicity.

- Increased incidence of hyperplasia, inflammation was observed in kidney, prostate, and urinary bladder at high dose.

Study no.:

— N103485

Sponsor Reference No: AT-21-08

Volume #, and page #: 1, 1-420

Conducting laboratory and location: —

Date of study initiation: May 13, 2004

GLP compliance: Not a final report

QA report: No

Drug, lot #, and % purity: Test article lot #233-2052A; Control (vehicle used for this study was Microsphere Diluent consisting of — carboxy methylcellulose, — Tween 20, and — NaCl in water for injection) lot # 165-0812 CA

Methods

Doses: 25, 100 mg/kg/ month

Species/strain: Sprague-Dawley, —

Number/sex/group or time point (main study): 10/sex/group

Route, formulation, volume, and infusion rate: Subcutaneous; Each dosing formulation (placebo, test article and control article) was prepared by the Sponsor Monitor by injecting 3.8 mL of Microsphere Diluent into the vial of placebo, test or control article (to yield a total volume of 5 mL; diluent contains: — CMC; — Tween 20; — NaCl). The powder was dispersed in the diluent by tapping, agitating and inverting each vial for a target time of 1 to 2 minutes, resulting in a suspension concentration of approximately 240 mg microspheres/mL for test and control articles and 248 mg/mL for the placebo. The volume of Sc injections were 0.35 and 1.4 mL for 25 and 1000 mg/kg, respectively

Satellite groups used for toxicokinetics or recovery: None

Age: Approximately 7 weeks

Weight: Males: 190-280 gms (approximately); Females: 145-192 gms (approximately)

Sampling times: TK analysis not done

Unique study design or methodology (if any): The rats were randomly assigned to four groups (10 rats/sex/group). Two dose groups received the test article with enhanced impurity levels (maintained at controlled room temperature during Alkermes stability study S 115). One group received a control article (stored frozen during Alkermes stability study Si 15) and another group received placebo (stored frozen). Due to the number of animals in this study, the dosing of the male rats began one day prior to female rats such that each sex had a respective

Day 1. Dosing was via subcutaneous injection once monthly over the course of 3 months (90 days). Dosing occurred on Days 1, 30 and 58. Due to a labeling error a significant protocol deviation was made, the control and the placebo groups were renamed.

Male animals numbered 101 to 110 were not included in statistical analysis and were designated as Treatment Group 1, as the males in this group were potentially treated with more than one test material. Data for this group were reported but not included in the comparative analysis. Female animals numbered 151 to 160 were designated as the Medisorb® Naltrexone Control group. Male animals numbered 201 to 210 and female animals numbered 251 to 260 were designated as Medisorb® Placebo group. The assignments for Groups 3 and 4 remain unchanged. Statistical analyses were performed by comparing all groups, except Male Treatment Group 1, to Group 2 (Medisorb® Placebo).

Experimental Design:

Group Number/ Description	Dose Volume (mL/kg)	Target Dose Concentration Naltrexone/Microspheres	Total Target Monthly Active Dose (mg/kg/dose)	Animal Numbers	
				Male	Female
2- Medisorb® Placebo	1.4	0 mg/mL/248 mg/mL ^a	NA	201-210	251-260
1- Medisorb® Naltrexone Control	1.4	76 mg/mL/240 mg/mL	100	101-110	151-160
3- Medisorb® Naltrexone	0.35	76 mg/mL/240 mg/mL	25	301-310	351-360
4- Medisorb® Naltrexone	1.4	76 mg/mL/240 mg/mL	100	401-410	451-460

a. Amendment 1

Observations, times, and Results:

Mortality: All animals were observed twice a day for morbidity/mortality. Animal #203 was found dead on Day 11. Macroscopic findings noted at necropsy included enlarged kidneys, enlarged urinary bladder and dilatation of the ureters. The pattern of lesions in the urinary tract was indicative of urinary tract obstruction as the cause of death.

Clinical signs: Animals were observed once daily for clinical signs. The clinical observation that was considered related to treatment were the swellings of the injection sites (injection sites 1, 2, or 3). The swellings were noted a few days following the administration of the test material in all groups (Placebo, Naltrexone control and Naltrexone 25 and 100 mg/kg). Swelling at some of the injection sites was not observed in various animals over the entire course of the study. However, swelling in at least one of the injection sites for all animals was identified at gross necropsy.

Body weights: Body weight was measured pretest and weekly after the initiation of the treatment. There was no significant change in group mean body weight noted during the course of the study attributable to naltrexone administration. All animals gained weight

throughout the course of the study; however, male animals in Group 1 (Naltrexone control), Group 3 (Naltrexone 25 mg/kg) and Group 4 (Naltrexone 100 mg/kg) did not gain weight at the same rate as the animals in the Placebo Group (Group 2). This trend was not apparent in the female group mean body weights.

Food consumption: Food consumption was noted qualitatively, no test article related changes in food consumption were observed.

Ophthalmoscopy: Not done

EKG: Not done

Hematology: All rats were fasted at least 12 hours prior to sampling for coagulation, hematology, and serum chemistry determinations. Blood samples were obtained from all surviving rats once pretest, once during Week 1, on Day 29, and on Day 90 prior to necropsy. Rats were anesthetized by utilizing a mixture of carbon dioxide/oxygen prior to blood collection from the retro-orbital sinus. Animals scheduled for necropsy on Day 90 were bled for coagulation parameters from the abdominal aorta or vena cava. The tubes contained EDTA as an anticoagulant for blood samples collected for hematology.

Small and frequently statistically significant decreases (compared to female placebo group results) in red cell counts, hemoglobin concentrations, and hematocrit results were frequently noted in the treated groups of females, though this trend was not noted in results from the treated groups of males. The differences between female placebo and treated female groups in red cell counts, hemoglobin concentrations, and hematocrit results were small in relation to variability that is expected in these parameters in untreated Sprague-Dawley rats. These hematology differences also did not show dose-dependency, nor did they increase in magnitude as more doses were administered. Biological relevance of this finding is not known.

Hematology and coagulation parameters measured or calculated were as follows:

Erythrocyte count (RBC)	Reticulocyte count (RET%)
Hemoglobin (HGB)	Platelet count (PLT)
Hematocrit (HCT) (calculated)	Total Leukocyte count (WBC)
Mean corpuscular volume (MCV)	WBC differential (absolute)
Mean corpuscular hemoglobin (MCH)	Prothrombin time (PT) ^a
(calculated)	Activated partial thromboplastin time
Mean corpuscular hemoglobin concentration	(APTT) ^a
(MCHC) (calculated)	
Differential leukocyte count = neutrophils, lymphocytes, basophils, eosinophils and monocytes	

a. Scheduled necropsy only.

Clinical chemistry: All rats were fasted at least 12 hours prior to sampling for coagulation, hematology, and serum chemistry determinations. Blood samples were obtained from all surviving rats once pretest, once during Week 1, on Day 29, and on Day 90 prior to necropsy. Rats were anesthetized by utilizing a mixture of carbon dioxide/oxygen prior to blood collection from the retro-orbital sinus. The serum was separated from the whole blood by centrifugation. Sodium citrate was used as the anticoagulant for coagulation parameters.

Serum chemistry measured included the following:

Alanine aminotransferase (ALT)	Albumin/globulin ratio (calculated) (A/G)
Aspartate aminotransferase (AST)	Cholesterol (CHOL)
Creatinine (CREA)	Triglycerides (TRIG)
Urea Nitrogen (BUN)	Glucose (GLU)
Alkaline phosphatase (ALP)	Sodium (NA)
Gamma-glutamyl transferase (GGT)	Potassium (K)
Bilirubin (TB)	Chloride (CL)
Total protein (TP)	Calcium (total)(CA)
Albumin (ALB)	Phosphorous (PHOS)
Globulin (calculated) (GLOB)	
Lactate dehydrogenase (LDH)	

No meaningful changes in the clinical chemistry parameter were noted.

Urinanalysis: Urine samples were collected overnight before scheduled clinical pathology blood collection during pre-study and on Day 90. In addition to the overnight collection on Day 90, an attempt was made at necropsy to aspirate any available urine from each animal’s bladder. The following parameters were evaluated:

Qualitative Evaluations		Quantitative Evaluations
pH	Occult Blood (OB)	Volume (UVOL)
Color (COL)	Urobilinogen (UBG)	Specific Gravity (SPG)
Appearance (APPR)	Ketones (KET)	
Urine Protein (UPR)	Bilirubin (BIL)	
Urine Glucose (UGL)	Sediment ^a	

a. Sediment Parameters = White Blood Cells (WBC), Red Blood Cells (RBC), Casts, Epithelial cells (EPI), Mucus (MUC), Sperm (SPRM), Bacteria (BACT), Yeast (YST), Amorphous sediment (AMOR), and Crystals (CRYS).

No meaningful changes in the urine analysis parameter were noted.

Gross pathology: All surviving rats were necropsied on Day 90. Complete necropsies were performed according to facility standard operating procedures on all rats that died during the study. All rats were fasted overnight for a minimum of 12 hours. Each necropsy included examination of external surface of the body; all orifices; the cranial,

thoracic, abdominal and pelvic cavities and their contents; and collection of all tissues. Nodules were observed in at least one of the injection sites of all animals, including all Medisorb® placebo group rats, with the exception of one Group-3 female Medisorb® Naltrexone rat. The incidence of nodules at the injection sites was slightly less for the Day 1 injection sites indicating some dissolution of the injected material occurred with time. Other gross observations were considered to be spontaneous findings unrelated to treatment, or in the instance of an eye lesion, secondary to retro-orbital bleeding. One male rat (#203) died on Study Day 11 with macroscopic findings of enlarged kidneys (due to pelvic dilatation), an enlarged urinary bladder, and dilatation of the ureters. Death was attributed to an incidental urinary tract obstruction.

Incidence Summary of Macroscopic Findings:

Group: Number in group:	-- Males --				-- Females --			
	2 ^a	1 ^b	3 ^c	4 ^d	2 ^a	1 ^e	3 ^c	4 ^d
Examined/No remarkable findings ...	0	0	0	0	0	0	1	0
CAVITY, ABDOMINAL								
FLUID	1	0	0	0	0	0	0	0
Total:	1	0	0	0	0	0	0	0
EYE								
ENLARGED	0	0	0	0	0	0	0	1
DISCOLORATION(S)	0	0	0	2	1	0	0	1
Total:	0	0	0	2	1	0	0	2
INJECTION SITE 1								
NODULE	9	6	2	6	9	8	0	4
Total:	9	6	2	6	9	8	0	4
INJECTION SITE 2								
NODULE	9	8	8	10	10	10	8	10
Total:	9	8	8	10	10	10	8	10
INJECTION SITE 3								
NODULE	9	10	10	10	10	10	9	10
Total:	9	10	10	10	10	10	9	10

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- a- Medisorb Placebo
- b- Potentially treated with more than one test material
- c- Medisorb Naltrexone, 25 mg/kg
- d- Medisorb Naltrexone, 100 mg/kg
- e- Medisorb Naltrexone Control, 100 mg/kg

Organ weights: No organ weight data was measure or submitted

Histopathology: Adequate Battery: Yes

Peer review: Yes

The following tissues were collected and fixed in 10 percent neutral-buffered formalin, except eyes, testes and marrow smears. Eyes and testes were preserved in Bouin's fixative. Bone marrow (from sternum) was fixed in methanol.

Tissues Collected at Necropsy	
Animal identification (collection only)	Ovaries
Adrenal glands	Pancreas
Aorta - Thoracic	Parathyroid gland(s)
Bone and bone marrow* (from sternum)	Pituitary gland
Brain	Prostate gland
Cecum	Rectum
Colon	Salivary glands (submaxillary)
Duodenum	Sciatic nerve
Epididymides	Seminal vesicles
Esophagus	Skeletal muscle (thigh)
Eyes with optic nerve	Skin (inguinal)
Gross lesions (if any)	Spinal cord (3 levels)
Heart	Spleen
Ileum (with Peyer's Patch)	Stomach
Injection site #1	Testes
Injection site #2	Thymus
Injection site #3	Thyroid gland
Jejunum	Tongue
Kidneys	Trachea (anterior and carina)
Liver	Urinary bladder
Lungs (all lobes)	Uterus (plus Cervix)
Lymph nodes – mandibular and mesenteric	Vagina
Mammary glands (inguinal)	

* Bone marrow (sternum) smears were prepared from all rats euthanized at scheduled necropsy. Hematology findings did not warrant examination of the smears.

Microscopic findings were graded semi-quantitatively using the following scale: a score of 1 (minimal) represented a barely detectable lesion unlikely to be of biological significance; a score of 2 (mild) represented a lesion likely to be subclinical and/or to have minor functional significance; a score of 3 (moderate) represented a lesion likely to have clinical significance; and a score of 4 (marked) represented a lesion approaching maximal in extent for the lesion in the organ.

Inflammation at injection sites was graded primarily on size; however, because of the limitations for size assessment microscopically, the macroscopic measurements of the size of the nodules may be more precise. Chronic inflammation was used as a diagnostic term at injection sites when the reaction was primarily fibrosis and lacking the mononuclear cell component present in granulomatous inflammation. Degeneration of the myocardium was used for lesions characterized by degeneration/necrosis of myofibers with the accumulation of a mixed population of cells primarily mononuclear cells. Inflammation at the injection sites occurred with about equal frequency and severity in the Medisorb® Placebo group and Medisorb® Naltrexone groups; therefore, inflammation at injection sites was attributed to a cellular response to the polymer microspheres of the Medisorb® Placebo. Increased incidence of hyperplasia, inflammation was observed in kidney, prostate, and urinary bladder at high dose.

Summary of Histopathological Findings:

Controls from group(s): 2 Tissues With Diagnoses	Animal sex: Dosage group: No. in group:	-- Animals --				Affected --			
		-- Males --				-- Females --			
		Ctls ^a	1 ^b	3 ^c	4 ^d	Ctls ^a	1 ^b	3 ^c	4 ^d
		10	10	10	10	10	10	10	10
EPIDIDYMS	Number examined:	10	10	0	10				
INFILTRATION, MONONUCLEAR CELLS	Average severity:	0	1	0	0				
ASPERMIA	Average severity:	1	0	0	0				
		0.4	0.0	0.0	0.0				
EYE	Number examined:	10	10	0	10	10	10	0	10
CATARACT	Average severity:	0	0	0	2	1	0	0	2
		0.0	0.0	0.0	0.6	0.3	0.0	0.0	0.6
ATROPHY, RETINA	Average severity:	0	0	0	2	1	0	0	1
		0.0	0.0	0.0	0.7	0.4	0.0	0.0	0.4
DYSPLASIA, RETINA	Average severity:	0	0	0	0	0	1	0	0
		0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
HEART	Number examined:	10	10	0	10	10	10	0	10
DEGENERATION, MYOCARDIUM	Average severity:	4	6	0	4	1	0	0	2
		0.5	0.8	0.0	0.4	0.1	0.0	0.0	0.2
INFLAMMATION, EPICARDIUM	Average severity:	1	0	0	0	0	0	0	0
		0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ILEUM	Number examined:	10	10	0	10	10	10	0	10
INJECTION SITE 1	Number examined:	10	10	0	10	10	10	0	10
INFLAMMATION, GRANULOMATOUS, SUBCUTANEOUS	Average severity:	9	8	0	8	8	8	0	4
		2.4	1.4	0.0	1.5	2.3	1.8	0.0	0.9
INFLAMMATION, CHRONIC, SUBCUTANEOUS	Average severity:	0	0	0	1	0	1	0	0
		0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0
INFLAMMATION, MIXED CELL, DERMIS	Average severity:	1	0	0	0	0	0	0	0
		0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
INJECTION SITE 2	Number examined:	9	10	0	10	10	10	0	10
INFLAMMATION, GRANULOMATOUS, SUBCUTANEOUS	Average severity:	8	9	0	10	10	10	0	10
		2.7	2.3	0.0	2.9	3.0	3.0	0.0	2.9
INFLAMMATION, CHRONIC, SUBCUTANEOUS	Average severity:	0	2	0	0	0	0	0	0
		0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
INJECTION SITE 3	Number examined:	9	10	0	10	10	10	0	10
INFLAMMATION, GRANULOMATOUS, SUBCUTANEOUS	Average severity:	9	10	0	10	10	10	0	10
		3.1	3.1	0.0	3.2	3.0	3.0	0.0	3.3
INFLAMMATION, MIXED CELL, DERMIS	Average severity:	0	1	0	1	0	0	0	0
		0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0
JEJUNUM	Number examined:	10	10	0	10	10	10	0	10
MINERALIZATION, LYMPHOID FOLLICLE	Average severity:	0	0	0	1	0	0	0	0
		0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
KIDNEY	Number examined:	10	10	0	10	10	10	0	10
NEPHROPATHY	Average severity:	5	6	0	5	1	1	0	2
		0.5	0.6	0.0	0.5	0.1	0.1	0.0	0.2
CYST	Average severity:	0	1	0	0	0	0	0	0
		0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
DILATATION, PELVIS	Average severity:	2	0	0	1	0	0	0	0
		0.4	0.0	0.0	0.2	0.0	0.0	0.0	0.0
INFLAMMATION, PELVIS	Average severity:	0	0	0	1	0	0	0	1
		0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.1
HYPERPLASIA, PELVIC EPITHELIUM	Average severity:	0	0	0	1	0	0	0	0
		0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
MINERALIZATION	Average severity:	0	0	0	0	1	1	0	0
		0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0
INFARCT	Average severity:	0	0	0	0	0	1	0	0
		0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
PROSTATE	Number examined:	10	10	0	10				
INFILTRATION, MONONUCLEAR CELLS	Average severity:	5	5	0	4				
		1.0	0.8	0.0	0.7				
INFLAMMATION, MIXED CELL	Average severity:	1	0	0	1				
		0.2	0.0	0.0	0.3				
HYPERPLASIA	Average severity:	1	0	0	3				
		0.2	0.0	0.0	0.5				
URINARY BLADDER	Number examined:	10	10	0	10	9	10	0	10
HEMORRHAGE	Average severity:	1	0	0	0	0	0	0	0
		0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
INFLAMMATION	Average severity:	1	0	0	2	0	0	0	0
		0.1	0.0	0.0	0.4	0.0	0.0	0.0	0.0
HYPERPLASIA, TRANSITIONAL EPITHELIUM	Average severity:	0	0	0	1	0	0	0	0
		0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0

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Toxicokinetics: Not done

2.6.6.4 Genetic toxicology

No genotoxicity studies for naltrexone and/or the PLG (excipients) were done by the Sponsor. The Sponsor has referenced the genetic toxicology data submitted for the Revia NDA (18-932) and provided a review of the publicly available literature as support for this NDA. The data for naltrexone is summarized below:

Naltrexone has been tested in a wide range of in vivo and in vitro genetic toxicology tests including bacterial systems, mammalian cells, tissue culture assays, and chromosome alterations. Naltrexone yielded negative results in all of the primary tests performed except in the *Drosophila*, in which an increase (2-3x over controls) in recessive lethal frequency was observed. This increase in excessive lethal frequency occurred at a concentration of naltrexone (10 mg/mL) which is well below the human therapeutic dose. The significance of this finding in a nonmammalian invertebrate species is unclear. Naltrexone produced negative results in all of the following gene mutation assays: Ames test, WP2 Reverse Mutation, Ade 3 Forward Mutation, Host-mediated Assay, Mouse Lymphoma Assay and Ruman tymphoblast Assay. In the in vivo chromosome alteration studies, naltrexone (at a dose up to 900 mg/kg administered via gavage to rats) produced no effect on clastogenicity and mitotic inhibition in bone marrow cells either upon acute or subacute (5 days) exposure. In the heritable translocation assay in germ cells (mouse sperm cells), Naltrexone doses up to 1030 mg/kg/day, p.o., were administered to male mice for a period of 7 weeks. When drug treated males were mated with unexposed females, naltrexone failed to increase the incidence of semi sterility or sterility in the F₁ male embryos. In secondary tests, naltrexone yielded results described as weak, non specific DNA damage in repair tests with *E. coli* and WI-38 cells, positive results in urine analysis for methylated histidine residues, and inconsistent results on sister chromatid exchange analysis.

The genotoxic potential of the PLG, [redacted] was examined in studies on [redacted] support that NDA application. [redacted]

[redacted] they did not submit patent certification for this NDA.

2.6.6.5 Carcinogenicity

There were no carcinogenicity studies submitted to the NDA. The Sponsor has referenced the results from carcinogenicity studies with naltrexone that were submitted in support of the Revia NDA. The study results for naltrexone are summarized below:

F 344 rats (50 males, 50 females/group) were treated via the diet with naltrexone at 30 and 100 mg/kg/day for 2 years. No serious toxicity was observed in this study and no adverse drug related influence on survival occurred. The trend analysis adjusted for mortality revealed a significant positive dose related increase in the incidence of vascular origin tumors for both males and females. However, this positive dose response relationship was due to small increases in tumors for males and females in the high dose group only. When the high dose rats were compared with those of matched controls using Fisher Exact Test, there were no statistically significant differences. The trend analysis adjusted for mortality also showed a statistically weak positive dose response relationship for testicular mesotheliomas in males. These tumors were generally late occurring and did not contribute significantly to the overall mortality of treated groups. The interpretation of these results is uncertain. Use of trend tests or historical comparisons increases the power to detect a difference in incidence of relatively rare events, but it also increases the frequency of false positive results. To examine these results further they were compared with a large series of published control studies. Only tumors of vascular origin had an incidence that was outside the upper limit of the incidence range seen in the historical studies (6% vs. 4% historical incidence). The low incidences and uncertain significance of the tumors that appeared in the Naltrexone rat study, coupled with their late occurrence and the lack of a similar finding in the mouse carcinogenicity study, diminishes the biological relevance of these findings. It is concluded, in accord with the recommendations of the 1960 NIDA Conference (9/3/90) that considered the issue, that the small increase in the incidence of tumors observed in naltrexone treated rats is insufficient to characterize the compound as a carcinogen.

Naltrexone was also evaluated in a 2-year carcinogenesis bioassay in B6C3F mice sponsored by the National Institute on Drug Abuse. For the carcinogenesis assay 50 mice/sex/group were treated with 0, 30, or 100 mg/kg naltrexone in feed. Gross lesions developed in 52% of controls, 44% of low-dose animals and 40% of high-dose animals after week 55. The overall survival rates for male mice were 74%, 70%, and 70% for the control, 30, and 100 mg/kg groups, respectively. The corresponding survival rates for female mice were 64%, 78%, and 82%. The most prevalent neoplasms for all groups included liver tumors, lymphomas, and lung neoplasms. However, these findings were not clearly related to dose or drug treatment.

The Sponsor's submission notes that PLG has been tested in a carcinogenicity study.

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However, the Sponsor has not provided patent certification that would be required to reference the findings to support this NDA, nor do they have right of reference to the data. As described in the Sponsor's

2.6.6.5 Reproductive and developmental toxicology

There were no reproductive toxicity studies submitted with this NDA. The Sponsor is referencing the reproductive toxicology studies for naltrexone that were submitted in support of the Revia NDA.

The summaries of reproductive toxicity findings that follow were derived from the Sponsor's submission. The Sponsor, in turn, summarized the findings from the NDA reviews for Revia that have been made publicly available (Sponsor provided patent certification for NDA 18-932 (Revia). The actual study reports were not re-evaluated for this NDA application.

Segment I - Fertility and General Reproduction Study of Naltrexone. Naltrexone was administered via gavage to — Rats as performed in the — the doses tested were 0, 20, 60, and 200 mg/kg/day. One female rat died (1/30) after 12 days of drug administration (high dose). The dam show clinical signs noted as “thin in appearance” and ‘tiptoe’ walk for approximately one week prior to death. Necropsy findings indicated signs of hemorrhage/brown color in the lungs, pitted surface in the heart, and enlargements in the thymus, mediastinal lymph nodes, and caecum. In addition, a membrane-encased mass of soft, coagulated, white, opaque material was found to fill the thoracic cavity and encased the lungs and heart. No other F₀ generation male or female rats died during the study. Dose related increases in incidences of salivation urination, urine-stained abdominal fur and/or ungroomed coat, and vocalization were seen both in male and female rats. In males, there was a dose related increase in excreted seminal plugs and hyperactivity/hypersensitivity. Females showed evidence of chromorrhinorrhea. Postmortem findings obtained at scheduled necropsy for both male and female rats indicated no drug related lesions.

The average body weight gain for F₀ generation male rats was slightly inhibited at high dose beginning on week 2 and continuing throughout the entire period of drug administration. In females, there was no change in mean body weight gain observed. No change in the physical behavior of F₀ male rats which mated, the average number of days in cohabitation, the average testes and epididymes weights or the average ratios of testes

and epididymes weight to body weight was observed for F₀ generation male rats administered naltrexone. For F₀ generation female rats, there was a significant increase (7-20%) in the incidence of pseudopregnancy at high dose compared with the vehicle. The "incidence of mating not resulting in pregnancy" was also increased at this dose level. Pregnancy occurred for 9/10 female rats (vehicle) mated by naltrexone treated male rats, and for 7/10 female rats (high dose, naltrexone) mated by vehicle control male rats. No change in estrous cycling, incidence of female rats which mated or the average number of days in cohabitation was found in the F₀ female rats after naltrexone administration. Naltrexone treatment resulting in a significant increase in the incidence of dams with resorptions and the average number of resorptions per litter. The incidence of dams with any resorptions were 3 (27.3%), 3 (21.4%), 8 (66.7%), and 8 (80%) for vehicle, low, mid, high dose of naltrexone, respectively. The corresponded average resorptions per litter were 0.3, 0.2, 1.2, and 2.6. All resorptions were determined to have occurred early in gestation. No F₀ generation females resorbed all implantations.

Natural delivery data showed one low dose and one mid dose dam (mated with vehicle control) each had no surviving litter beyond day 2 postparturition. This finding may have been either a drug-mediated effect on maternal behavior (poor maternal care, e.g. failing to remove placentas and umbilical cords from live born pups) or one secondary to pup mortality. Administration of naltrexone to dams mated with vehicle control males significantly increased the absolute incidence of stillborn pups. Although not significant the average number of stillborn pups per litter, and the incidence of delivering stillborn pups were also increased for treated dams mated either with treated or vehicle control males. So was the incidence of absolute stillborn pups mated with vehicle control dams with treated males. The incidence of dams delivering stillborn pups in vehicle control (both males and females untreated), and 0, low, mid and high dose naltrexone groups was 2 (14.3%), 1 (8.3%), 1 (7.7%), and 3 (27.3%), respectively with an average of 0.1, 0.2, 0.1 and 0.3 stillborn pups per litter. In these same respective groups, the incidence of stillbirth was 2 (1.4%), 2 (1.4%), 1 (0.6%), and 3 (2.0%). One (11.1%) of the vehicle control dams (mated with males) and three (42.9%) of the treated dams (mated with vehicle control males) delivered stillborn pups; with averages 0.1 and 0.9 stillbirths per litter, respectively; the incidence of stillborn pups in these same respective groups was 1 (0.8%) and 6 (6.2%). Administration of naltrexone to dams mated either with vehicle control or naltrexone treated males also resulted in a slight, but statistically non-significant decrease in pup weight/litter during the lactation period, i.e. day 1-21 post parturition. No change in pup survival 7 day and 21 day post parturition (excluded those two dams that had no surviving litter beyond day 2 post parturition), length of gestation, implantations, dams surviving parturition, litter size, and pup sex ratio from birth to day 21 post parturition was observed. Gross litter observations from birth to day 21-post parturition of F₁ generation pups indicated weak appearance, cold to touch and not nursing were drug related physical signs/behavior noted for litters of dams administered naltrexone. No other litter observations noted were considered drug related since they occurred infrequently and/or were not dose-dependent. Postmortem physical findings revealed no drug related signs were observed either for pups which died during the first 21 days post parturition or for those sacrificed on day 21 post parturition.

Post parturition or for those sacrificed on day 21 post parturition. Development and reproductive capacity of F₁ generation pups were measured at pre weaning, post weaning and in the sexual maturation of the pups.

Pre weaning reflex and physical development (Birth to Day 21 post-parturition showed that the developmental time course of acoustic startle at which at least 50% of the pups in each litter exhibited such a reflex as slightly, but significantly delayed ($p < 0.05$) for maternal high dose group litters (high dose F₀ females x Vehicle F₀ males) as compared to maternal vehicle dose group (Vehicle F₀ females x Vehicle F₀ males). This observation correlated with the slight, spontaneous decrease in average body weights for these pups. The average day post parturition that acoustic startle was observed for at least 50% of the pups per litter in maternal dose group were about 13 days. The developmental time courses of pinna unfolding, eye opening, surface righting, and pupil constriction in the F₁ generation pups were not affected by naltrexone administration to the F₀ generation rats.

Post weaning observations (beginning on Day 22 post parturition) showed that two F₁ generation female rats died during the post-weaning period. One maternal low dose group rat died on day 22 post weaning and had yellow—orange stained fur in the inguinal area, necropsy findings revealed lungs were moderately hemorrhagic and the stomach was gas filled, thin walled and distended; the pyloric region of the stomach was filled with red brown exudates and the large and small intestines were gas filled; the liver was white in color, spotted and the median lobe contained a dark area. The other F₁ female died on day 8 post weaning. No gross lesions were observed at necropsy. Physical signs described in the ante mortem findings include the most frequently seen and possibly drug related physical signs was cloudy eye(s); the next frequently observed but non dose related signs were eroded/ulcerated cornea, enlarged eye(s), ptosis, alopecia, and lesion. Other signs occurred either infrequently or were observed in only one rat. The sponsor attributed the observed cloudy eye(s), ptosis enlarged salivary glands, eroded/ulcerated cornea, and chromodacryorrhea to the SDA virus infection. No dose-related postmortem physical signs were observed for F₁ generation males and females either at scheduled sacrifice or at Caesarean sectioning. The most common signs which occurred in either equal or lower incidences in the treated groups as compared with the vehicle were lung lesions (numerous dark red nodules or several darkened red brown areas on the surface or lobes of the lung), unilateral/bilateral hydronephroses and opaque and/or enlarged eye(s). Other infrequent, non dose-related signs included liver abnormalities (super numerary medial lobe, and numerous firm, shiny, tan colored masses on the liver) spleen with a firm, shiny, tan colored mass, abdominal cavity with small, firm, red-brown colored masses adhered to adipose tissue, enlarged testis, lesions of uterus or ovaries, and a small, red-colored mass in subcutaneous fat of the inguinal area. Sexual maturation of F₁ generation as measured by the age at which either the testes descended or the vagina was patent indicated that administration of Naltrexone did not influence such study parameters.

Behavioral testing was done using the following tools: a) inclined plane-motor coordination of approximately six-week old F₁ generation and female rats, when tested

on the inclined plane, was not influenced by administration of Naltrexone to the F₀ generation rat; b) Watermaze- Learning (evaluated by the average # of trials to criterion and the average # of errors per trial), short term retention (measured by the average latency to escape the maze on trial 2 of day 1 of testing), long term retention (measured by the difference in the # of trials to criterion and the error rate per trial from day 1 to day 2, and the latency on trial 1 of day 2 of testing), and swimming ability of approximately 10-wk old F₁ generation rats were not influenced by Naltrexone administration to the F₀ generation rats. At weaning body weight of F₁ generation male and females from all treated group significantly decreased (upto 50 % compared to vehicle group). The body weight gain for F₁ males at post weaning period was with all dose groups was significantly higher than that of the females. Reproduction capacity of the F₁ males and females were generated by a) Mating and fertility analysis- F₁ generation rat's incidence of rats which mated, the incidence of mating that resulted in pregnancy and the average # of days in cohabitation for the F₁ generation male and female rats were not affected by administration of Naltrexone to the F₀ generation rats.

Although average absolute testes and epididymes weight was heavier in the maternal dosage group as compared to the vehicle control, this difference was not dose-related. No difference of relative testes and epididymides weight was observed among the six maternal dosage groups. b) Caesarean sectioning Data - F₁ generation dam's uterine content after C-section were not affected by Naltrexone administration to the F₀ generation. The average of corpora lutea, implantations, resorptions, litter sizes, the average number of live fetuses per litter and the incidence of gross external fetal malformations were similar for F₁ generation female rats in all maternal dose groups. Although the incidence of resorption tended to increase slightly in the high maternal dosage groups, this increase did not reach statistical significance. The incidence of rats with one or more resorption sites per litter was increased with dose. Resorptions were mainly early. No changes in body weights and sex ratios were observed in the F₂ generation fetuses.

Segment II reproductive toxicology study was carried out by the administration of Naltrexone (0, 20, 60, and 200 mg/kg/day. p.o. via gavage) in pregnant (SD) rats (dosing duration: days 6 through 15 of presumed gestation). Observations include analysis of different parameters in dams (body weight, physical signs/general appearance, mortality, signs of abortion, death or natural delivery, pregnancy, and placement of implantations, early and late resorptions, # of corpora lutea, gross lesions upon necropsy) and litters (body weight, gross external variations, soft tissue anomalies, and skeletal variations). Pregnancy occurred in 24 (96%), 24 (96%), 22 (88%) and 24 (96%) rats given 0(vehicle), 20, 60, and 200 mg/kg/day. dosages of Naltrexone respectively. Two pregnant rats in the high dose group died (These deaths occurred on either day 11 or day 13 of gestation following six or eight daily dosages, respectively, and were the direct results of tracheal intubation which occurred as a secondary result of adverse drug-related effects (vocalization, hyper reactivity, violent twisting of the body during intubation and/or excess salivation). Fluid in the thoracic cavity was found upon necropsy and was suggested to be due to fluid aspiration by the animals while struggling

during intubation. Adverse clinic signs which were attributed to Naltrexone administration to rats consists of vocalization, hyperreactivity, violent twisting of the body during intubation, chromorhinorrhea, excess salivation, ptosis, and red discharge from mouth. Convulsions occurred in one high dose animal (1/24). As compared to the control, the dosages of Naltrexone significantly inhibited (average maternal body weight gain). The effect was dose related, reversible, and disappeared by day 20 of gestation. At caesarean-sectioning on day 20 of gestation, one mid dose rat (1/25) had parovarian cysts and one mid dose (1/25) and high dose rats (2/26) had dilated pelvis of one or both kidneys. Average number of corpora lutea, implantations, litter size, live and dead fetuses, resorptions and incidences of rats with resorptions, and rats with all implantations resorbing were similar and were not so different among the four dosage groups. So were the litter data including the average # of live fetuses, average fetal body weights per litter, dead or resorbed implantations per litter and the fetal sex ratio. No gross external fetal variations were observed except one high dose fetus (1/310) which had an ectopic tail. No significant dose related soft tissue variations were observed. One low dose fetus (1/152) had diaphragmatic lobe agenesis of the lung. One mid dose fetus (1/152) exhibited slight dilatation of the third ventricle. A slight, non dose-related increase in the incidence of dilated lateral ventricles was seen in the treated groups (0/153, 3/152, 1/152, and 2/148 fetuses for vehicle, low, mid and high). The slight to moderate dilatations of renal pelvis of one or both kidneys were found to occur equally in the vehicle and the treated groups. A tendency of incomplete ossification of the skull in the high dose group including parietal, interparietal, frontal, nasal, and supraoccipital was seen. The incidence, however, was low (1 to 2 fetuses out of 162 fetuses in the high dose as compared to zero in the other groups), the significance of the finding is not known. Other skeletal variations included one kyphosis (1/162) and 2 irregular shaped sternbrae (2/162) in the high dose group. The rest of the variations were seen in low incidence and occurred either equally in the vehicle and treated groups or at a lower incidence in the treated groups. Differences in fetal ossification site averages were neither dose dependent nor significant among the four dosage groups. These included cervical, thoracic, lumbar, sacra), and caudal vertebrae, sternbrae, xiphoid, true ribs, false ribs, carpals, metacarpals, tarsals, metatarsals, forepaw/ phalanges.

Segment II reproductive toxicity study of Naltrexone administered orally via stomach tube to New Zealand White Rabbits (dose: 0 (vehicle), 20, 60 and 200 mg/kg/day and duration: days 6 through 18 of presumed gestation) was conducted. Analysis of maternal parameters include physical signs and/or general appearance during prestudy period, and on day 0 of presumed gestation, physical signs, abortion, and/or viability during dosing period and post dosing period (days 19 through 29 of gestation, several times each day), body weight (weekly during acclimation, day 0 of gestation, daily during the dosing and post dosing periods).

Caesarean Sectioning at day 29 of gestation include analysis of maternal gross lesions, # and placement of implantations, fetuses, early and late resorptions, number of corpora lutea. In case of abortion, uterine contents and necropsy were recorded on the same day on which the abortion occurred. Fetal gross external variations, body weight, sex, visceral and skeletal variations, late resorptions were examined to the extent possible.

No deaths occurred during the study. Possible drug related non-dose dependent physical signs seen in the F₀ generation rabbits were soft/liquid feces, and alopecia. The drug effects were evident by an increase in either the number of days or the number of animals exhibiting such signs. Other signs observed which occurred either in equal or lower incidence as compared to the vehicle group were anorexia, red exudate, rales, excess lacrimation. Administration of Naltrexone at mid and high dose inhibited average maternal body weight gain during days 6 to 9 and 6 to 12 of gestation as compared with vehicle. The effect disappeared gradually and by the end of the study (day 29 of gestation) the drug treated groups actually tended to increase in average maternal body weight gain. Pregnancy occurred in all dosage groups more or less equally. One low dose group rabbit aborted an early resorption on day 22 of gestation. This rabbit had only one implantation site in utero and had a red exudate in the cage pan on day 20 of gestation. No adverse postmortem physical sign was observed for this rabbit. No rabbit naturally delivered a litter prior to schedule maternal caesarean sectioning. Administration of Naltrexone to dams produced a dose related increase, although not statistically significant, in resorptions. This was evident by an increase in the total # of resorptions, the average number of resorptions per litter, the incidence of rabbits with any resorptions and the incidence of rabbits with all implantations resorbing. The increase in resorptions was mainly due to early resorption and there existed a dose dependent effect for such early resorptions.

The average number of resorption per litter was 0.6, 0.8, 1.8, and 2.5; the incidence of rabbits with any resorptions was 4 (40 %), 8 (57.1 %), 6 (50.0 %) and 8 (61.5%); and the incidence of rabbits with all implantation resorbing was 0, 0, 1 (8.3%) and 1 (7.7%) in control, low, mid and high dose group respectively.

Administration of Naltrexone to pregnant rabbits did not affect the incidence of pregnancy, average number of implantation or average litter sizes. One dead fetus was present in a high dose group litter. Postmortem finding revealed no abnormalities for its developmental age.

Administration of Naltrexone to pregnant rabbits caused a dose-related increase although not statistically significant in the average percentage of dead or resorbed implantations per litter (5.6, 11.3, 25.6, and 31.9) for the vehicle, low, mid and high dosage groups, respectively. Maternal administration with Naltrexone did not affect either the fetal body weight or sex ratio.

In general, the of Naltrexone to dams did not result in either a dose related or statistically significant increase in fetal variations of any particular kind. However, at 200 mg/kg/day, there existed a tendency for the incidence of litters with fetuses with any variation, and the average fetuses with any variation per litter to increase as compared with the vehicle.

One or more variations were observed in 5 (6.7 %), 8 (10.1%), 1 (1.2%), and 12 (15.4 %) fetuses in vehicle, low, mid and high dosage group litters, respectively. Fetuses with any

variation observed per litter averaged 8.75, 9.95, 0.91 16.12 percent in the vehicle control, low, middle, and high dose group litters, respectively.

The host of variations observed included enlarged fontanelle, hydrocephalus, edema in the neck and the abdominal area, nyelocoele or meningocoele, dark or red colored lung enlarged and/or clear gall bladder, incomplete ossification of the parietals, parietals with small holes, fused sternbrae an unossified pubis.

Segment III perinatal and postnatal reproductive toxicity study with Naltrexone administered (orally via gavage) were done in (SD) female rats with 0, 10, 30, and 100 mg/kg/day. The animals were dosed from day 15 of gestation to day 21 post parturition. Necropsy was done at day 21 post parturition. Study parameters include evaluation of maternal (mating performance, body weight, physical signs during parturition, length of gestation, length of parturition, maternal behavior, and litter size, pup viability at birth, time of birth, and necropsy) and F₁ pups (viability, gross litter observations, litter count, physical signs including nursing behavior and gross external physical anomalies and dead pup recording (daily), examination for gross lesions and cause for moribund and dead pups, preservation of tissues with gross lesions for future evaluation).

Maternal observations include drug related slight to marked excess salivation seen in the high dosage group. Other minor clinical changes were swollen axilla and/or chest. No drug related gross lesions were noted at necropsy. The lesions observed included moderate to marked dilatation of the pelvis of one or both kidneys in all dose group and a perforated esophagus and left axillary region engorged with a red-brown material in one high dose dam, and a tiny fluid filled area in the left uterine horn in another high dose dam. A significant, dose related inhibition of average maternal body weight gain was evident three days after, drug administration (day 18 of gestation) and continued through day 20 of gestation. Maternal body weight change decreased with increased dose (averaged +34.6, +31.3*, +25.3** and +20.0** grams for days 15 to 18 of gestation and +59.6, +56.2, +52.4** and 45.4** grams for days 15 to 20 of gestation in the vehicle, low mid and high dose groups respectively (*=significant).

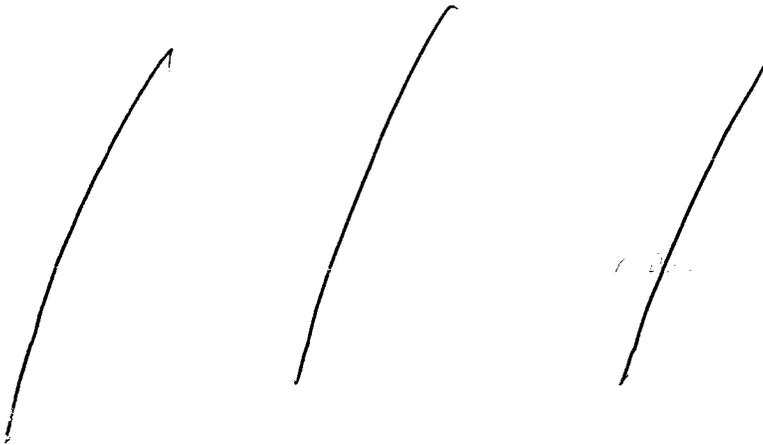
The dose-related decrease in average maternal body weight persisted to day 1 of lactation for dams administered with Naltrexone (283.7, 279.9, and 275.3 gms for vehicle, low, mid., and high dose respectively), thereafter dose-dependent increases in average maternal body weight gain from day 1 to 4 of lactation occurred (+6.5, +11.0, +12.0, and 13.2 gms respectively). Neither effect, however, was significant. Subsequent average maternal body weight gain during the remainder of the lactation period was similar for all dosage groups.

Administration of Naltrexone did not affect the incidences pregnancy, of dam surviving parturition, dams delivering pups, averages for implantation length of gestation, duration of parturition, or incidences of dams with stillborn or with one or more live pups and of dams with no pups surviving to day 21 post parturition. Thin appearance, not nursing, cold to touch, decreased maternal care, weak appearance and lesion occurred in a non dose related fashion.

No post mortem physical signs observed for stillborn pups, for pups which died during the 21-day post parturition period or pups sacrificed on day 21-post parturition were related to Naltrexone administration to the F₀ generation dams.

The two postmortem findings observed were a missing tail, in a low dose stillborn pup and a unilateral “pop-eye” (due to SDA viral infection) in a mid dose young.

Effects of the Vehicle Formulation. The sponsor submitted an evaluation of the safety for the PLG vehicle that was based on the



However, in the absence of adequate patent certification or right of reference, to the PLG data, only Segment I and III reproductive toxicity study it is recommended that the data for the above mentioned toxicity studies should be provided for labeling purpose. It is my opinion that the deficiencies noted should not prevent the approval of this NDA; however, a post marketing commitment should be acquired for documentation of safety in regards to the reproductive toxicity of the vehicle for the Vivitrex.

2.6.6.6 Local tolerance

Study title: An Acute Local Tolerance Study of Vehicles Administered Subcutaneously to Rabbits.

Key Findings:

- Grossly visible red and purple/blue foci corresponded microscopically to hemorrhage. Focal scabs, degeneration and inflammation of skeletal muscle and occasional fragmentation of hair-shafts were observed. No test material was discernible within the skin/subcutaneous sections.
- vehicles 1 and 2 appeared to induce a mixed inflammatory response in the subcutis of most injection sites. The subcutaneous inflammation was slightly more marked in the vehicle 2 than with vehicle 1.

Study Number:

— A 074-716

Sponsor Reference No: AT-03-09

Conducting Laboratory:

The aim of this study was to determine the local tolerability of two different formulations of — vehicle. The test articles were — vehicles 1 —
 — Tween® 20, — NaCl) and — vehicles 2 — carboxymethyl cellulose,
 — Tween® 20, — NaCl. Twelve male New Zealand White rabbits were randomly distributed by weight stratification to three treatment groups. Each animal received single subcutaneous injections of the control (saline) and test materials in distinct sites on the dorsal area that was previously shaven. A fourth site (uninjected) was identified and used as a comparative control. The day of dosing was designated as Day 1 of the study. The animals were evaluated for changes in clinical signs and body weight, and the injection sites were examined and graded for any reaction to treatment. Four animals at each of 3 timepoints (3, 5 and 8 days) were sacrificed and the subcutaneous injection site areas removed and processed for histopathological examination.

Experimental Design:

Group	Number of Males	Treatment	Dose Vol. (ml)	Day of Sacrifice
1	4	Control (untreated)	-	3
		Control (saline)	2	
		vehicle 1	2	
		vehicle 2	2	
2	4	Control (untreated)	-	5
		Control (saline)	2	
		vehicle 1	2	
		vehicle 2	2	
3	4	Control (untreated)	-	8
		Control (saline)	2	
		vehicle 1	2	
		vehicle 2	2	

No animals died and no test article-related clinical signs or body weights were noted during the study. A few instances of injection site discoloration (bluish color) were observed post dosing. A few incidences of mostly slight to mild erythema were seen in injection regions of a few rabbits on some days post dosing at the saline control and — vehicle 1 and 2 injection sites. However, there was no clear evidence that the erythema was test article-related. Similarly, there were a few occurrences of localized enlargements that resembled edema following dosing at the saline control and — vehicle 1 and 2 injection sites.

Gross necropsy observations at Day 1 and their microscopic correlates showed red/tan, pink or purple/blue foci were observed in 3 of 4 saline control, 2 of 4 — vehicle 1 and 3 of 4 — vehicle 2 injection sites. In all microscopically represented foci,

the correlate was hemorrhage or congestion most probably associated with the trauma of the subcutaneous injection procedure.

Scabs on the surface of the epithelium, focal epidermal necrosis, fragments of follicular structures within the dermis, focal inflammation of the skeletal muscle and hemorrhage were generally attributed to the trauma of injection for saline and [redacted] vehicle 1 and 2 injection sites. A small focus of mild necrosis was present in the subcutaneous tissue of one section from a [redacted] vehicle 1 site, it was located on the margin of the injection site, bordered by a zone of fibrin and more centrally by a broad area of moderate hemorrhage, suggesting a further traumatic procedure-related pathogenesis. Minimal to mild subcutaneous inflammation characterized primarily by macrophages and lymphoid cells with occasional heterophils was present in 2 of 4 saline control, 3 of 4 [redacted] vehicles 1 and 3 of 4 [redacted] vehicle 2 injection sites.

No gross necropsy findings were observed in Day 5 necropsied animals. Microscopically observed scabs, inflammation or degeneration of skeletal muscle and hemorrhage were minimal to mild and attributed to the trauma associated with subcutaneous injection. Mild inflammation was present in the subcutaneous tissue of 3 of 4 sites each in [redacted] vehicles 1 and 2. One [redacted] vehicle 2 site had moderate subcutaneous inflammation. In addition, subcutaneous cyst-like borders/spaces suggestive of fascial planes yet co located with inflammation and occasionally be in circular in configuration were noted in 1 of 4 [redacted] vehicle 1 and 2 of 4 [redacted] vehicle 2 sites. Mature collagen fibers and inflammatory cells were scattered on both sides of the margins. There was no appreciable evidence of recent fibrosis. The cyst-like borders/spaces were composed of a single layer of mature fibroblast-like cells juxtaposed by scattered foamy macrophages.

Gross necropsy observations at Day 8 consisted of red or red/blue foci were observed at 1 of 4 saline control and 2 of 4 [redacted] vehicle 2 sites. As seen at the Day 3 and 5 sacrifices, focal degeneration and inflammation of skeletal muscle and hemorrhage was noted at the Day 8 sacrifice. Mild subcutaneous inflammation was present in 2 of 4 [redacted] vehicles 1 and 3 of 4 [redacted] vehicle 2 sites with the remaining [redacted] vehicle 2 site having moderate subcutaneous inflammation. All subcutaneous inflammation sites, except for 1 [redacted] vehicle 2 sites, were accompanied by cyst-like borders/spaces.

Study title: An Acute Local Tolerance Study of [redacted] Formulations [redacted] and [redacted] Administered Subcutaneously to Rabbits.

Key Study Findings:

- A local enlargement was seen immediately following injection of [redacted] formulations and lasting through Day 8 of the study may due to the presence of the test material.
- The gross observable findings of red or white foci corresponded to hemorrhage and/or the presence of the [redacted] formulations. Microscopically, the two

formulations within the subcutaneous sites were indistinguishable. At Day 4, the local response consisted of heterophilic inflammation. However, by Day 8, it was compounded by granulomatous inflammation in which increased numbers of giant cells, focal/multifocal fibrosis and fibroblast proliferation were all present. Compression of preexisting collagen seen in 11 of 12 sites at Day 4 persisted and was present in all 12 sites at Day 8. New collagen formation that was limited to 5 of 8 [redacted] formulation sites at Day 4 had progressed and was present in all 8 [redacted] formulation injection sites at Day 8. The degree of new collagen formation was indistinguishable between sites and never graded higher than mild.

- Subcutaneous administration of [redacted] and [redacted] was well tolerated in rabbits. There was no clinically detectable local irritation at the injection sites. Macroscopic and microscopic responses at the subcutaneous injection sites appeared similar with both polymers.

Study Number:

[redacted] A071-716

Sponsor Reference No: AT-07-01

Conducting Laboratory: [redacted]

The study was performed to determine the local tolerability of two [redacted] formulations [redacted] and [redacted] when administered subcutaneously to rabbits. Eight New Zealand White male rabbits were assigned to two treatment groups as shown in the table below.

Experimental Design:

Group	Number of Males	Treatment	Dose (mg)	Dose Vol. (ml)	Day of Sacrifice
1	4	Control Untreated	-	-	4
		Vehicle Control	0	1	
		[redacted]	100	1	
		[redacted]	100	1	
2	4	Control Untreated	-	-	8
		Vehicle Control	0	1	
		[redacted]	100	1	
		[redacted]	100	1	

Each animal received single subcutaneous injections of the [redacted] vehicle carboxymethyl cellulose, [redacted] Tween®20, [redacted] NaCl) and both [redacted] formulations in distinct sites on the dorsal area that was previously shaven. A fourth site (uninjected) was identified and used as a comparative control. The day of dosing was designated as Day 1 of the study. The animals were evaluated for changes in clinical signs and body weight, and the injection sites were examined and graded for any reaction to treatment. Four animals at each of 2 time points (Days 4 and 8) were sacrificed and

the subcutaneous injection site areas removed and processed for histopathological examination.

No animals died and no treatment-related clinical signs, effects on body weights or treatment-related erythema were observed. Enlargement or swelling at the local injection sites was seen following a subcutaneous administration of — formulations that was attributed to the physical presence (1 ml volume per site) of the test material.

At Day 4, microscopic evaluation of the subcutaneous injection sites showed a moderate inflammatory response (subacute) for both — formulations that were indistinguishable from each other. The sites contained a slight preponderance of heterophils with a few scattered giant cells. Seven of 8 — vehicle injection sites showed compression of preexisting collagen and 5 of 8 — formulation sites showed minimal to mild new collagen formation. At Day 8, the inflammatory response (still indistinguishable between formulations) had progressed. It was now characterized as granulomatous and contained an increased number of giant cells with focal/multifocal fibrosis and fibroblast proliferation in the surrounding subcutaneous tissue. Residual collagen compression and new collagen formation (graded as 1 minimal and 11 mild) were present in all injection sites. There were no test article-related adverse effects noted during the study.

Grossly observed red foci, when microscopically represented, corresponded with hemorrhage. White foci observed in all — formulation — and — injection sites corresponded to the respective injected formulations. The formulations and their respective tissue reactions were microscopically indistinguishable in character and intensity within each of the two sacrifice time points. For both — formulations the inflammation on Day 4 contained a heterophilic component with an occasional giant cell. At Day 8, there was no appreciable reduction in the amount of test material. The surrounding subcutaneous tissue reaction was represented by condensation of connective tissue, focal/multifocal fibrosis and fibroblast proliferation, neovascularization and increased numbers of giant cells (granulomatous inflammation). Heterophils continued to predominate.

Inflammatory Response Summary:

Response	Day 4			Day 8		
	Vehicle Control	/	/	Vehicle Control	/	/
Inflammation	+1 ¹ , +2, +2			+2, +2		
Inflammation, subacute		+3, +3, +3, +3	+3, +3, +3, +3			
Inflammation, granulomatous				+2, +2	+3, +2, +3, +3	+3, +3, +3, +3

¹ Each entry represents the highest grade of a finding for each animal. The absence of an entry indicates no inflammation.

In several injection sites, the cystic spaces containing — formulations were surrounded by a satellite zone containing additional cysts which resembled dilated lymphatic vessels. The — vehicle control sites at Day 8 also had subcutaneous

cyst-like borders/spaces but they tended to be more singular and compressed. vehicle was not apparent microscopically. The accompanying inflammation was characterized as granulomatous in 2 of 4 sites however the relationship of inflammation to vehicle control may have been confounded by "foreign body" induced inflammation as the result of hair shaft fragments introduced at the time of injection. vehicle control inflammatory response at both time periods was generally more focal, limited and generic as compared to the more pronounced heterophilic and later more granulomatous response induced by formulations.

Study Title: Development of Drug Delivery System.

Key Study Findings:

- None of the test article implant sites demonstrated any toxic effects such as necrosis and or unusual reactions which are uncommon to the controls.
- No unusual clinical signs have been observed in any of the treated or other control animals through Day 365, study completion. The body weight data indicated that all treated and control animals have gained weight during the course of the study.

Study Number:

Toxikon Project: 89G—0102

Sponsor Reference No: AT-07-02

Conducting Laboratory:

Sprague-Dawley male rats were given implants (1x5 mm disc) and subcutaneous injections (100 mg/ ml and 0.3 ml was injected) of Test Article I or Test Article II

No test article related mortality or unusual clinical observations were noted during the 365-day study.

The animals were treated once on Day 0. Each animal received a subcutaneous injection of test material and a subcutaneous implant of the same test material. The injection was made in the right flank, and the implant in the left flank. The injection site on each animal was tattooed immediately prior to injection to permanently mark the site throughout the life of each animal. The suspending medium for the microspheres was prepared and sterilized by the Sponsor. The microspheres were suspended (100 mg/ml) immediately prior to treatment.

To implant the disc each animal was anesthetized with xylazine (5 mg/kg) IM plus ketamine (40 mg/kg) IM. The flank was clipped and scrubbed with iodine solution. A one inch longitudinal incision through the skin will be made along the midline of the flank. A subcutaneous pocket adequate for the disc (1 mm x 5 mm) was formed to the left side of the incision. The disc was placed in the pocket, and the incision was closed

with surgical staples. Each control animal was injected (suspending medium without microsphere and sham operated (without implant)).

Experimental Design
Test Article I

Group	Group Number	Number of Animals	Animal Numbers	Day of Sacrifice
Treated	1	6	1-6	Day 1
	2	6	7-12	Day 2
	3	6	13-18	Day 4
	4	6	19-24	Day 7
	5	6	25-30	Day 14
	6	6	31-36	Day 21
	7	6	37-42	Day 28
	8	6	43-48	Day 60
	9	6	49-54	Day 121
	10	6	55-60	Day 184
	11	6	61-66	Day 365
Control	12	2	67-68	Day 1
	13	2	69-70	Day 2
	14	2	71-72	Day 4
	15	2	73-74	Day 7
	16	2	75-76	Day 14
	17	2	77-78	Day 21
	18	2	79-80	Day 28
	19	2	81-82	Day 60
	20	2	83-84	Day 121
	21	2	85-86	Day 184
	22	2	87-88	Day 365

2 Test Article II

Group	Group Number	Number of Animals	Animal Numbers	Day of Sacrifice
Treated	1	6	1-6	Day 1
	2	6	7-12	Day 2
	3	6	13-18	Day 4
	4	6	19-24	Day 7
	5	6	25-30	Day 14
	6	6	31-36	Day 21
	7	6	37-42	Day 28
	8	6	43-48	Day 60
	9	6	49-54	Day 121
	10	6	55-60	Day 184
	11	6	61-66	Day 365

The tissue responses such as chronic inflammation, layer of mild tissue granulation around implant sites and phagocytic response were common reactions observed in histopathology. Similarly, the histopathological examination of implants of the test article spheres of _____ exhibited granulation of tissues at implant sites,

fibrosis and inflammation of tissues similar to the earlier test article. The observed tissue reaction from test article disc of Poly lactide-CO-glycolide and spheres of Poly lactide-CO-glycolide) demonstrated similar tissue responses, as narrated earlier. The control implant sites for discs and spheres showed tissue responses similar to the test articles. The histopathology has indicated the presence of bland local responses to implantation and subcutaneous injection of both test articles with no evidence of undue necrosis or inflammation.

Study Title: Local Tolerance Study of Medisorb® Naltrexone in Rabbits Following Single Subcutaneous and Intramuscular Injections.

Key study findings:

- The subcutaneous injection of both the test material and the control material microspheres resulted in predominate microscopic findings of granulomatous inflammation and a fibrous response enveloping the residual test or control material and active fibroblast hyperplasia at Day 8 and at Day 30. At Day 30, the test material site had minimal to slight lymphoplasmacytic inflammation in four of five animals.
- The intramuscularly administered test and control materials were located in all of the microscopic sections with the exception of the control material section. The predominant finding was slight to moderately severe granulomatous inflammation and occasional minimal fibroblast hyperplasia. At Day 8, minimally to moderately severe muscle degeneration necrosis (regeneration for a single control material site), minimal to slight edema, and minimal hemorrhage were also noted. At Day 30, the test material sites of all five animals had minimal to slight muscle regeneration and minimal to slight lymphoplasmacytic inflammation.
- Microscopically, the residual test and control material at both the subcutaneous and intramuscular sites consisted of spherical structures that sometimes contained refractile material in the processed section. The subcutaneous and intramuscular test material sites and one intramuscular control material site also had fragmentation of the spherical structures.
- Under the conditions of this study, subcutaneous injections of Medisorb® Naltrexone, Medisorb® Placebo Microspheres, and Diluent and the intramuscular injection of Medisorb® Naltrexone and Medisorb® Placebo Microspheres to male albino rabbits were well tolerated.

Study Number:

— 6403-118
Sponsor Reference No: AT-07-01

Conducting Laboratory

The study was done to assess the local tolerance for Medisorb® Naltrexone when administered as a single dose by subcutaneous and intramuscular injections to rabbits. Male Hra:(NZW)SPF rabbits (approximately 16 weeks old and weighing from 3.053 to 3.529 kg; were used for the study. The test material (Medisorb® Naltrexone; Lot No. 190-0292A) contained approximately 322 mg Medisorb® Naltrexone (approximately 114 mg naltrexone based on 35.5% drug load) and the control material (Medisorb® Naltrexone Placebo Microspheres; Lot No. 191 -2738A; without active drug), contained approximately 333 mg placebo microspheres. The vehicle Diluent; Lot No.) was composed of carboxymethylcellulose, Polysorbate 20 (Tween 20), and sodium chloride. Test and control materials were administered as subcutaneous and intramuscular injections and the vehicle was administered subcutaneously according to the following design:

Experimental Design:

Group	Number of Animals ^a	Treatment							
		Subcutaneous Injection ^b				Intramuscular Injection ^c			
		A	B	C	D	Dose Volume	Left Side	Right Side	Dose Volume
1	5	UC	PD	MPM	MN	2 mL/site	MPM	MN	2 mL/site
2	5	UC	PD	MPM	MN	2 mL/site	MPM	MN	2 mL/site

UC Untreated Control

PD Diluent

MPM Medisorb® Naltrexone Placebo Microspheres (Medisorb® Placebo Microspheres)

MN Medisorb® Naltrexone

a Group 1 was sacrificed on Day 8 and Group 2 was sacrificed on Day 30.

b Each animal received each subcutaneous treatment (identified as Sites B, C, and D); an untreated control site (identified as Site A) was included in the evaluation.

c Each animal received both intramuscular treatments (identified as left and right injection sites).

Doses were selected by the sponsor based on the maximum dose and volume expected to be delivered at an individual site in proposed clinical trials. Two different dose routes, subcutaneous and intramuscular injection, were assessed in this study as both routes of administration will be evaluated in clinical trials.

Clinical observations were conducted at 1-hour post-dose and once daily, thereafter. On Days 1 through 8 and on Days 10, 12, 14, 16, 18, 21, 23, 25, 28, and 30, injection sites were evaluated visually for evidence of local irritation and surface measurements were taken of any local enlargements to estimate the size of residual test or control materials. Five animals (Group 1) were sacrifice on Day 8 and the remaining five animals (Group 2) were sacrificed on Day 30. At necropsy, macroscopic observations were recorded for the

injection sites. The injection sites were collected, digitally photographed, and examined microscopically.

Within a few days following subcutaneous administration of a 2 mL dose volume of test material, control material or vehicle, a localized enlargement was noted for some injection sites. Thereafter, these enlargements, in part, diminished in size over the 30 day study period. There were no erythematous reactions at any of the subcutaneous untreated control sites or vehicle (— Diluent) sites nor any localized enlargements with the exception of one vehicle-treated site that had a measurement of 20 x 15 mm (length x width) on Day 1 and one vehicle- treated site that had a maximum measurement of 40 x 40 mm (length x width) on Days 1 and 2.

Subcutaneous injection of the control material (Medisorb® Placebo Microspheres) resulted in site enlargements that ranged maximally from 20 x 30 mm to 40 x 35 mm (length x width) in two of five animals in each group. These enlargements were present in two animals in Group 1 from Days 1 and 4, respectively, until the Day 8 sacrifice and were present in two Group 2 animals from Day 1 through Day 10. A very slight erythematous reaction was noted at a single control material subcutaneous site in a Group 2 animal on Days 3 through 8.

Subcutaneous injection of the test material (Medisorb® Naltrexone) resulted in site enlargements that ranged maximally from 10 x 15 mm to 40 x 35 mm (length x width) in four animals in each group. For Group 1, enlargements were present in three animals from Day 3 or 4 until the Day 8 sacrifice and in one animal on Day 1 and Days 4 through 8 (not present on Days 2 or 3 in this one animal). Enlargements were also present in four Group 2 animals from Day 1, 2 or 4 until the Day 30 sacrifice. A very slight erythema reaction was also observed at the test material site of two of the five animals in Group 1 and in four of the five animals in Group 2. These erythema reactions were still observed at the Day 8 necropsy in Group 1 and cleared in all animals in Group 2 by Day 16.

Intramuscular injection of the test or control materials did not result in any injection site enlargements. A very slight erythematous reaction at the test material injection site was observed in one Group 1 animal on Day 8. Two animals in Group 2 had a very slight erythematous reaction at the control material injection site from Day 3 or 5 through Day 10. There were no unexpected or meaningful effects on body weights during the study. Individual microscopic findings by group and individual anatomic pathology data are in At necropsy, residual test and control materials were observed at all dose sites. Besides the macroscopic notations of light and/or red focus/foci at the injection sites, the only other macroscopic observation was focal hemorrhage in the posterior lumbar-sacral area in two animals. Microscopic examination of these lumbar-sacral areas of focal hemorrhage concluded that the macroscopic change observed at necropsy was not related to the test material, control material, or vehicle but is probably related to the anesthetic injections of xylazine/ketamine at the time of treatment.

There were no microscopic findings for any of the untreated subcutaneous control sites with the exception of minimal granulomatous inflammation in the subcutis of one animal

at Day 30. A minimal to slight amount of fibroblast hyperplasia in four of five animals was noted at Day 8 at the subcutaneous injection site treated with the — Diluent vehicle. At Day 30, minimal fibroblast hyperplasia and minimal granulomatous inflammation of the subcutis was noted in one of five animals.

The subcutaneous injection of both the test material and the control material microspheres resulted in predominate microscopic findings of granulomatous inflammation and a fibrous response enveloping the residual test or control material and active fibroblast hyperplasia at Day 8 and at Day 30. Occasional minimal to slight edema and hemorrhage was also noted for test and control material sites. At Day 30, the test material site had minimal to slight lymphoplasmacytic inflammation in four of five animals.

The intramuscularly administered test and control materials were located in all of the microscopic sections with the exception of the control material section for one animal necropsied at Day 30. This one control site had been located at the time of necropsy but was not present microscopically. The predominant finding was slight to moderately severe granulomatous inflammation and occasional minimal fibroblast hyperplasia. At Day 8, minimally to moderately severe muscle degeneration necrosis (regeneration for a single control material site), minimal to slight edema, and minimal hemorrhage were also noted. At Day 30, the test material sites of all five animals had minimal to slight muscle regeneration and minimal to slight lymphoplasmacytic inflammation.

Microscopically, the residual test and control material at both the subcutaneous and intramuscular sites consisted of spherical structures that sometimes contained refractile material in the processed section. The subcutaneous and intramuscular test material sites and one intramuscular control material site also had fragmentation of the spherical structures. Under the conditions of this study, subcutaneous injections of Medisorb® Naltrexone, Medisorb® Placebo Microspheres, and — Diluent and the intramuscular injection of Medisorb® Naltrexone and Medisorb® Placebo Microspheres to male albino rabbits were well tolerated.

Study title: Chronic Local Tolerance Study of Medisorb® Naltrexone in Rabbits Following Single Subcutaneous and Intramuscular Injections

Key study findings:

- Under the conditions of this study, subcutaneous and intramuscular administrations of Medisorb® Naltrexone, Medisorb® Naltrexone Placebo Microspheres, and — Diluent to male rabbits were well tolerated.
- The treatment related effects were limited to the reactions at the local injection sites. Minimal tissue effects (ie degeneration/regeneration of skeletal muscle, fibroplasia/fibrosis, and occasional inflammation) were seen at the sites treated with the — Diluent and was primarily limited to the Day 8 sacrifice.

- Macroscopic and microscopic findings were similar for subcutaneous and intramuscular injections of the Medisorb® Naltrexone and Medisorb® Naltrexone Placebo Microspheres.

Study no.:

5403-119
Sponsor Reference No: AT-21-04

Volume # and page #: 1 and 1-486

Conducting laboratory and location: _____

Date of study initiation: September 18, 2000

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: The test material (Medisorb® Naltrexone; Lot No. 192-2350A) and the control material (Medisorb® Naltrexone Placebo Microspheres; Lot No. 198-1740A) each contained approximately 470 mg of microspheres per vial. When reconstituted with the vehicle (_____ Diluent), each vial contained approximately 281 mg microspheres/mL.

Formulation/vehicle: The vehicle (_____ Diluent; Lot No. F03A8a) was composed of _____ carboxymethylcellulose _____ polysorbate 20, _____ sodium chloride, and water.

Methods**Doses:**

Based on the 34.7% drug load and the target dose volumes (2 or 3 mL), the nominal naltrexone dose for the Medisorb® Naltrexone sites were 195 and 293 mg/dose for the subcutaneous and intramuscular sites, respectively. Test, placebo control, and vehicle control materials were administered as subcutaneous and intramuscular injections according to the following design:

Experimental Design:

Study Design Table							
Groups ^a	Number of Animals	Treatment					
		Subcutaneous Injection ^b			Intramuscular Injection ^b		
		Site A	Site B	Dose Volume ^c	Left Side	Right Side	Dose Volume ^c
1, 3, 5, 7, 9	5/group	D	MN	2 mL/site	D	MNPM	3 mL/site
2, 4, 6, 8, 10	5/group	D	MNPM	2 mL/site	D	MN	3 mL/site

D — Diluent
 MNPM Medisorb® Naltrexone Placebo Microspheres
 MN Medisorb® Naltrexone

a Five animals from each treatment regimen were sacrificed at each of the designated times (Days 8, 30, 89, 150, and 240).

b Each animal received two subcutaneous treatments (A and B) at sites distant to each other and two intramuscular treatments, one on each leg.

c Target dose volumes.

Study design:

Each animal received two separate subcutaneous bolus injections. The duration of each subcutaneous injection was approximately 5 seconds. The shaved back of each animal was divided into two areas. Treatment sites (A and B) were rotated among the two locations to avoid any potential location site bias.

Each animal also received two separate intramuscular injections into the thigh muscle (biceps femoris) of the hind leg (one in the right leg and one in the left leg; see study design table). The duration of each intramuscular injection was approximately 5 to 15 seconds.

Treatment. Before dosing and as needed thereafter, the hair within and surrounding the intended injection site on both thighs and at the dorsal region of the rabbit was clipped with an electric clipper using care to avoid nicks or cuts. Before dose administration on Day 1, the animals were anesthetized to effect by intramuscular injections of xylazine and ketamine in the paralumbar musculature. The target dose volumes for the subcutaneous and intramuscular administrations were 2 and 3 mL/injection, respectively. Given the target dose volumes for the subcutaneous and intramuscular injections and the drug load for the Medisorb® Naltrexone, the target dose delivered based on the concentration of 281 mg/mL were as follows:

Subcutaneous dose based on a target dose volume of 2 mL/injection site

- Medisorb® Naltrexone Microspheres (195 mg naltrexone)
- Medisorb Naltrexone Placebo Microspheres

Intramuscular dose based on a target dose volume of 3 mL/injection site

- Medisorb® Naltrexone Microspheres (293 mg naltrexone)
- Medisorb Naltrexone Placebo Microspheres

Clinical observations were conducted on Day 1 predose and at approximately 1 hour postdose and once daily thereafter. Body weights were recorded on Day 1 (prior to dosing) and weekly thereafter. Injection sites were evaluated visually for local irritation (redness) and any site enlargement before dosing on Day 1, daily during Week 1, and twice weekly thereafter. Groups 1 and 2 were sacrificed on Day 8, Groups 3 and 4 were sacrificed on Day 30, Groups 5 (four animals) and 6 and one Group 9 animal were sacrificed on Day 89, Groups 7 and 8 were sacrificed on Day 150, and Groups 9 (four animals) and 10 and one Group 5 animal were sacrificed on Day 240. At the respective sacrifice interval, animals were euthanized, subjected to an abbreviated necropsy examination, and macroscopic observations were recorded for the injection sites. The injection sites were collected, photographed, and examined microscopically.

Results:

All animals survived to their respective scheduled sacrifice interval. Abnormal observations included malocclusion, missing teeth (associated with the malocclusion in one animal), clear oral discharge, few or liquid feces, skin scab formation (on ears, digits, thoracic region, abdomen, dorsal area, or scrotum), red skin (dorsal-thoracic region), yellow- or brown-stained hair coat (head, limb, paws, sacral region, or tail), low food consumption, and small tissue mass on ear. One animal (F07659) in Group 9 (originally designated for sacrifice on Day 240) was euthanized on Day 89 due to poor health (malocclusion missing teeth).

Diluent (Subcutaneous Injection Sites)

There were no abnormal or irritation effects at these injection sites other than one animal in Group 8 that had a hemorrhage at the subcutaneous injection site on Days 2 through 5. This was attributed to the injection trauma caused by the dose administration procedure.

Medisorb® Naltrexone (Subcutaneous Injection Sites)

Very slight erythematous reactions in a total of four animals (one in Group 1 and three in Group 9) were observed at these injection sites. These reactions were only evident from Day 2 to Day 8. Local enlargements at the injection sites (attributed to the subcutaneous injection of the depot material) were clinically visible and/or palpable in 24 of the 25 injection sites. These enlargements tended to maintain their dimensional size for animals sacrificed up to Day 30, but then gradually decreased in size for animals sacrificed on Days 89 through 240.

Medisorb® Naltrexone Placebo Microspheres (Subcutaneous Injection Sites)

Very slight erythematous reactions in a total of five animals (one in Group 6, two in Group 8, and two in Group 10) were observed at these injection sites. These reactions were only evident from Day 2 to Day 8. Local enlargements at the injection sites (attributed to the subcutaneous injection of the depot material) were clinically visible and/or palpable in 21 of the 25 injection sites. As with the Medisorb® Naltrexone sites, these enlargements tended to maintain their dimensional size for animals sacrificed up to Day 30, but then gradually decreased in size for animals sacrificed at the later intervals.

Two animals (one in Group 2 and one in Group 6) had hemorrhage at the subcutaneous injection sites during the first week; this was attributed to the dosing procedure.

— Diluent (Intramuscular Injection Sites)

There were no abnormal effects at these injection sites.

Medisorb® Naltrexone (Intramuscular Injection Sites)

Very slight erythematous reactions in a total of five animals (two in Group 6 and three in Group 10) were observed at these injection sites. These erythematous reactions were seen on Day 2 only for all animals except for one where the reaction persisted to Day 4. There were no other findings at these sites.

Medisorb® Naltrexone Placebo Microspheres (Intramuscular Injections Sites)

Very slight erythematous reactions were seen in a total of two intramuscular sites (one in Group 7 and one in Group 9). These reactions were observed only on Days 2-3 or 2-4. Local enlargements at the injection sites were noted in three animals in Group 7 (Days 113 to 148) and in one animal in Group 9 (Days 116 to 151). These animals had no treatment-related clinical adverse effects.

Due to the diet ration offered to the animals each day, the animals maintained a nearly steady state condition in regards to body weight. Any weight losses that did occur intermittently during the study were not considered to be test or control material related.

The Medisorb® Naltrexone subcutaneous and intramuscular injection sites had a similar macroscopic appearance to the placebo formulation injection sites at all sacrifice intervals. The amount of residual test or placebo material at the treatment sites was notably less at the Day 240 sacrifice when compared to the earlier sacrifice intervals. This residual material generally had a light colored appearance (tan to white) at both the subcutaneous and intramuscular injection sites. There were also instances of red, dark or mottled focal areas at the subcutaneous and intramuscular injection sites with a slightly higher incidence of these findings which were associated with the subcutaneous injection of Medisorb® Naltrexone. The — Diluent subcutaneous and intramuscular injection sites had no macroscopic findings other than light focal areas at the subcutaneous injection site of two animals sacrificed on Day 150. This was not considered to be a treatment-related finding. There were no macroscopic findings in the untreated skin and muscle tissues.

Microscopically, the subcutaneous and intramuscular injection sites for Medisorb® Naltrexone had a similar appearance to those same sites treated with the placebo formulation. The Medisorb® Naltrexone and Placebo Microspheres were readily identified at each sacrifice interval as refractile and occasionally fragmented spherical structures that were generally surrounded by a variable fibrous response and granulomatous inflammation. The severity grade of the local inflammatory response at the Medisorb® Naltrexone and Placebo sites was initially low (minimal to moderate at 1-week postdose) and exhibited a peak reaction at 1- and 3-months postdose (minimal to moderately severe). The inflammatory response gradually lessened after 3 months at the

Medisorb® Naltrexone sites and after 5 months in the Placebo sites. By 8 months, the inflammatory response was either not existent or significantly less than those present at 5 months. Further, the residual materials were significantly degraded, in that, by the 8-month sacrifice, there was either no or very little microsphere material remaining (present as macrophage intracytoplasmic foreign material). The test and placebo subcutaneous injection sites often had some residual fibrous connective tissue present in close proximity to the remaining foreign material. At Day 240, other than minimal residual fibrous connective tissue at one placebo and one Medisorb® Naltrexone intramuscular injection site, there was no fibrosis present in the intramuscular sites. Fibrosis was also significantly less in the subcutaneous sites at Day 240 than at earlier intervals. Minimal to moderate hemorrhage was observed at some of the subcutaneous and intramuscular sites at the Day 8 and Day 30 sacrifice intervals. The hemorrhage was attributed to the dose administration procedure. Minimal to moderate degeneration/regeneration of skeletal muscle was also observed at some of the Medisorb® Naltrexone and placebo intramuscular injection sites (and at one placebo subcutaneous injection site) at the Day 8 and Day 30 sacrifices. A few of the test and placebo intramuscular injection sites also had an adipose tissue infiltration into the skeletal muscle at Days 150 and 240.

Microscopic findings at the Diluent subcutaneous and intramuscular injection sites were very limited and consisted of minimal degeneration/regeneration of skeletal muscle, minimal to slight fibroplasias/fibrosis, and occasional inflammation which primarily occurred at the Day 8 sacrifice.

Study title: Local Tolerance and Pharmacokinetic Evaluation of Medisorb® in Dogs Following Repeated Intramuscular Injections

Key study findings:

- Repeated intramuscular administrations of approximately 1144 mg Medisorb® - (approximately equivalent to 394mg naltrexone/dose) to dogs produced a clinically evident and reversible swelling at the injection site.
- All dogs were healthy and there were no other treatment-related clinical effects.
- The microscopic findings at treatment sites consisted of chronic active inflammation, progressing to granulomatous inflammation and accompanied by fibrosis.
- Recovery from these local reactions was progressive and was largely complete by 6 months after administration of the last dose.
- Administration of repeated doses of test formulation in closely adjacent intramuscular sites at intervals of 30 and 60 days resulted in some local accumulation of residual test material, however, no significant pathological differences in injection site reactions were observed when compared to the reaction elicited by single dose administration.
- The naltrexone pharmacokinetic profile was not different when repeated intramuscular dosing was performed at the same or at separate sites.

Study no.:

403-121

Sponsor Reference Number: AT-21-05

Volume #, and page #: 1, 1-366

Conducting laboratory and location:

Date of study initiation: January 29, 2001

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: 192-2950A, 192-2850A

Formulation/vehicle: The vehicle, Medisorb Diluent (carboxymethyl cellulose sodium polysorbate 20; NaCl), also identified as Diluent, Lot No. L02A9a.

Methods

Doses: 1144 mg Medisorb® (approximately equivalent to 394 mg naltrexone/dose), IM, in a 4 mL/dose volume.

Experimental Design:

Group	Number of Dogs	Dose Site	Dose Days	Sacrifice Days (No. of dogs)	Nominal Dose Level ^b (mg/dose)	Approximate Dose Volume (mL/dose)
1 ^a	7 ^c	separate	1 and 30	18 (1) ^d , 44 (3), 210 (2), and 301 (1) ^c	1144	4
2	7	same	1 and 30	18 (1), 44 (3), 210 (2), and 301 (1)	1144	4
3	6	same	1 and 60	74 (4) and 240 (2)	1144	4
4	6 ^c	same	1, 60, and 120	134 (4) and 301 (2)	1144	4

a Group 1 was a local tolerance control for Groups 2, 3 and 4; Group 1 was a pharmacokinetic control for Group 2.
 b mg/dose of microspheres (Medisorb®) the nominal active drug dose was approximately 394 mg (based on a drug load of 34.4%).
 c Effective 27 February 2001 (Day 29), Group 1 male H38446 was treated as a Group 4 dog (Dosing occurred on Days 1, 60, and 120 and the sacrifice took place on Day 301). However, due to computer limitations, all observations and dose administration for this dog were recorded under the original dog group designation (Group 1).
 d The animal sacrificed on Day 18 noted in Group 1 is animal number 1138464. It was moved to Group 1 from Group 4 to maintain consistent with the number of animals designated per Group stipulated in the original protocol. This change was made only to rebalance the experiment, as all animals up to Day 18 received the same treatment (injection on Day 1). This animal was not included in the pharmacokinetic studies.

Study design:

Thirty, approximately 7- to 9-month-old, male Beagles were used for this study. At initiation of dosing, the dogs were approximately 7 to 9 months of age with body weights ranging from 10.4 to 13.3 kg. Prior to initiation of dosing and as needed thereafter; the hair surrounding the intended injection site area was clipped with an electric clipper using care to avoid nicks or cuts. The site(s) of injection was identified and marked by a permanent marker. The two separate injection sites (opposite legs, one per dose) in the Group 1 dogs were identified by a mark. Dogs in Groups 2, 3, and 4 had each injection

site marked; the successive dosing for these dogs was in close proximity (not more than approximately 2 cm apart) to the previously injected site. Group 4 received a second and third dose on Days 60 and 120 respectively. The inlife observations and evaluations used to assess toxicity included mortality and moribundity checks, postdose clinical observations, body weights, and injection site observations. Plasma samples were collected for pharmacokinetic evaluation from animals in Groups 1 and 2 up to Day 120. When unexpected injection site swelling occurred after the first injection, an additional interim sacrifice with pathologic evaluation was conducted on two animals on Day 18. All other sacrifices were conducted as scheduled at 14 days and 6 months after the second or third dosing. An abbreviated gross necropsy was performed. Injection sites were isolated, examined, photographed, and preserved in formalin. Histopathology examination was performed on paraffin-embedded sections with hematoxylin and eosin stained slides of injection sites and untreated control sites. Pathological evaluation was based on the composite reaction involving the entire treated area for Groups 2, 3 and 4 where a series of 2 or 3 Medisorb® — injections were administered in closely adjacent sites.

Results:

Medisorb® — produced swelling at the dose sites of all dogs. There was some variability in the severity; however, the swelling was generally present within one to two weeks of dose administration and persisted for two to four weeks thereafter. A purulent discharge was noted from the second dose site (left leg) in the Group 1 dog H38441 on Day 36 (dosed administered on Day 30). Discharge was also noted for Group 4 animals 1-138460 and H38465 on Days 9 and 133, respectively. Bacterial cultures on aspirated fluid from the injection sites of dogs H38442 (Group 1) and H38453 (Group 2) isolated *Staphylococcus sciuri* and *Staphylococcus cohnii*, respectively. The bacteria are regarded as normal flora and were not considered pathogenic. Cultures on aspirated fluid from the injection site of H38445 (Group 1) did not isolate any bacterial colonies.

These data, in conjunction with the pathology findings, indicate that the swellings at the dose injection sites were sterile abscesses, resulting from local tissue reactions to the test material. In most instances, evidence of swelling was not observed at the time of subsequent dose injections in Groups 2, 3, and 4. The character of the injection site reactions was qualitatively comparable across groups.

Erythema was also noted, though infrequently, at the injection site of most dogs. It was characterized most often as very slight, though in some instances it was well defined. The character and frequency of the erythema finding was comparable across groups and consistent with treatment-related reactions at the dose injection sites.

Two Group 3 dogs (1-138457 and H38459) had limited use of their right hind legs. This was attributed to treatment, but was only noted on one or two occasions and is regarded as an atypical finding.

On Day 25, H38442 (Group 1) had a convulsion lasting approximately 2 minutes and was ataxic and hypoactive during the postictal period. The dog's behavior returned to normal within 2 days and was unremarkable thereafter. The underlying cause of the seizure is unclear.

There was no effect of Medisorb® → on body weights.

The naltrexone pharmacokinetic profile following repeated intramuscular administrations of Medisorb at different sites or at the same or closely spaced injection sites in dogs (Groups 1 and 2, respectively) was similar. For groups 1 and 2, the naltrexone C_{max} values were 49.2 ± 17.6 and 55.2 ± 10.4 ng/ml, respectively, after a single dose. After the group 1 animals received their second dose in the contralateral leg, C_{max} was 69.9 ± 16.4 ng/ml. Animals that received their second dose in close proximity to the first dose (Group 2) had a C_{max} of 60.3 ± 27.5 ng/ml after the second dose. For Groups 1 and 2, naltrexone AUC was 410 ± 70.1 and 392 ± 42.9 ng.day/ml, respectively. Following the second dose, AUC was 428 ± 43.1 and 318 ± 70.6 ng.day/ml for Groups 1 and 2, respectively. Pharmacokinetic parameters (C_{max} and AUC) were comparable following Dose 1 and Dose 2 and no trend (increase or decrease) in the data following the second naltrexone injection was noted for either group. The data also indicate that repeated dosing at the same or different sites has little if any effect on the exposure to naltrexone in the dog. On Day 30, prior to the second dose, naltrexone levels were either not detectable (<0.2 ng/ml) or not greater than 1.3 ng/ml. Following the second dose, naltrexone levels were generally not detectable after about 7 weeks post-dose. The concentrations of 6β-naltrexol were all below or near the limit of quantitation of the assay at all points sampled during the study.

Animals H38452 and H38464 were sacrificed on Day 18 to investigate unexpected adverse reactions seen clinically at the injected muscle site. Injection site findings related to the test material consisted of extensive chronic-active inflammation and an attempt to contain the administered foreign material. At this stage of tissue reaction development, the containment was a poorly formed capsule characterized by a variably thick layer of macrophages, fibroplasia, neovascularization, and edema. Associated with this cellular reactivity and elsewhere within the injection site was inflammation consisting of neutrophils, eosinophils, macrophages, plasma cells, lymphocytes, and multinucleated giant cells. Neutrophils were often present within the foci of the residual depot material. The injection site also had minimal to slight degeneration/regeneration of skeletal muscle near the test material. There were no findings for the untreated muscle of either dog.

At Day 44 terminal sacrifice, three dogs (H38440, H38441, H38442) from Group 1 and three dogs (H38447, H38448, H38449) from Group 2 were sacrificed. Group 1 dogs had received the nominal microsphere dose of 1144 mg in the right thigh muscle on Day 1 and 1144 mg in the left thigh muscle on Day 30. Group 2 dogs received the same dose of test material on the same days as Group 1; however, the second injection for Group 2 dogs was administered at approximately the same site as the first injection in the right thigh muscle.

The macroscopic findings for the injection sites in Groups 1 and 2 were similar to those of the Day 18 interim sacrifice. In general, the left leg injection site of Group 1 (Day 30

dose only) and the right leg injection site of Group 2 (Days 1 and 30 dose) were microscopically similar to the injection sites of the interim sacrifice. The right leg injection site of Group 1 had more fibrous connective tissue present, less granulocytic cells, and a greater proportion of phagocytic cells. There was minimal skeletal muscle degeneration in the untreated muscle tissue of one Group 2 dog.

At Day 74 terminal sacrifice, four dogs (H38454, H38455, H38456, H38457) from Group 3 were sacrificed. Group 3 dogs had received the nominal microsphere dose of 1144 mg test material in the right thigh muscle on Day 1 and a second injection was administered on Day 60 at approximately the same site as the first injection.

In general, the cellular reaction around the area of the two injections had less granulocytic activity than the injection sites evaluated from the Day 18 and 44 sacrifices. Degeneration/regeneration of skeletal muscle continued to be present, and hemorrhage was present in two of the dogs. Fibroplasia also continued to be present. Structures resembling microspheres were found at all of the examined injection sites. Extensive granulomatous inflammation with a lymphoplasmacytic component was present. Like the other injection sites examined 14 days after injection, there were intralesional neutrophils in some animals. There were no findings for the untreated muscle of these dogs.

At Day 134 terminal sacrifice, four dogs (H38460, H38462, H38463, H38465) from Group 4 were sacrificed on. Group 4 dogs had received the nominal dose of 1144 mg test material in the right thigh muscle on Day 1, a second injection of 1144 mg test material on Day 60, and a third injection of 1144 mg test material on Day 120, all within close proximity of the first injection.

Macroscopic findings were similar to those at the previous terminal sacrifices, in which a raised area or swelling and pale was visible on the cut surface of the injection site. One dog also had an enlarged right inguinal lymph node and fluid accumulation at the injection site. Two dogs had microscopic evidence of a partial encapsulation of the microspheres, while the other two dogs had a much more extensive and active inflammatory cellular response (overall finding of chronic active inflammation for the injection site). The latter two dogs also had fibrosis at the injection site. The fibrosis may have been directed at the test material injected on Day 1 and 60. Structures resembling microspheres were found at all of the examined injection sites. Degeneration/regeneration of skeletal muscle continued to be present, and hemorrhage was present in one of the dogs. Like the other injection sites examined 14 days after injection, there were intralesional neutrophils in one of the dogs with an extensive active inflammatory response around the injected material. There were no findings for the untreated muscle of these dogs.

At Days 210 and 240 recovery sacrifices, two dogs (H38443 and H38444) from Group 1 and two dogs (H38450 and H38451) from Group 2 were sacrificed on Day 210. Group 1 dogs had received the nominal microsphere dose of 1144 mg in the right thigh muscle on Day 1 and 1144 mg in the left thigh muscle on Day 30. Group 2 dogs received the same dose of test material as Group 1 dogs; however, the second injection for Group 2 dogs

was administered at approximately the same site as the first injection in the right thigh muscle.

There were no macroscopic findings for the Day 1 right thigh injection site from the Group 1 dogs. Each of the Group 2 dogs with two injections in the right thigh had pale material present at the injection site. At the left injection site (dosed on Day 30) one Group 1 dog had pale material present and the other dog had tan raised areas. Structures resembling microspheres were found at two of the right injection sites (1 of 2 Group 1 dogs and 1 of 2 Group 2 dogs) and 1 of 2 left injection sites from Group 1. With progressive resolution of the injection sites, tissue alteration included very limited skeletal muscle degeneration/regeneration, persistence of a limited fibrous response (one right thigh injection site from each group), and fibrosis in one left thigh injection site. Inflammation was of the granulomatous type, with the most extensive inflammation remaining in the Group 2 right thigh injection sites. There were no macroscopic or microscopic findings for the untreated muscle tissue.

Two dogs (1138458 and H38459) from Group 3 were sacrificed on Day 240. Group 3 dogs had received the nominal microsphere dose of 1144 mg in the right thigh muscle on Day 1, and a second injection was administered on Day 60 at approximately the same site as the first injection.

Macroscopically, there was a tan area involving the cut surface of the right thigh injection site for one dog (H38459). There were no macroscopic findings for the right thigh injection site for the other Group 3 dog (H38458). The only microscopic finding was minimal granulomatous inflammation in the injection site skeletal muscle of each dog. There were no macroscopic or microscopic findings for the untreated muscle tissue.

At Day 301 recovery Sacrifice, one dog (H38445) from Group 1, one dog (H38453) from Group 2, and two dogs (H38461 and H38446) from Group 4 were sacrificed on Day 301. Group 1 dogs had received the nominal microsphere dose of 1144 mg in the right thigh muscle on Day 1 and 1144 mg in the left thigh muscle on Day 30. Group 2 dogs received the same dose of test material as Group 1 dogs; however, the second injection for Group 2 dogs was administered at approximately the same site as the first injection in the right thigh muscle. Group 4 dogs had received the nominal microsphere dose of 1144 mg test material in the right thigh muscle on Day 1, a second injection of 1144 mg test material on Day 60, and a third injection of 1144 mg test material on Day 120, all within close proximity of the first injection.

Macroscopically, all dogs had a pale area or pale material at the respective injection site for that treatment group. Microscopically, the examined sections of skeletal muscle at the injection site from the Group 2 dog were unremarkable. The Group 1 dog only had minimal skeletal muscle regeneration at the right (Day 1 dosed) injection site; however, the left (Day 30 dosed) injection site had minimal granulomatous inflammation often containing intracytoplasmic residual test material. The two Group 4 dogs had slight to moderate granulomatous inflammation and minimal to slight fibrosis at the injection site. There were no macroscopic or microscopic findings for the untreated muscle tissue.

Summary of Toxicokinetic Study Design in Dogs:

Group	N	Injection Site	Nominal Naltrexone Dose (mg/animal)	Dosing Study Days	Animal Number	Sampling Period (Study days)
1	7	Opposite leg	394	1 and 30	38440 - 38442	44
					38443 - 38445	120
					38446	26
2	7	Same injection leg/site	394	1 and 30	38447 - 38449	44
					38450, 38451, 38453	120
					38452	18
3	6	Same injection leg/site	394	1 and 30	No Plasma Sampling	
4	6	Same injection leg/site	394	1 and 30	No Plasma Sampling	

Summary of Pharmacokinetic Parameters of Naltrexone in Beagle Dogs Following Two 394 mg Intramuscular Injections of Medisorb-021 on Study Day 1 and 30 for Group 1 (Opposite Leg) and Group 2 (Same Site) (Study AT-21-05).

Group	Mean	Dose 1 (N=7)					Dose 2 (N=6)				
		T _{max} (day)	C _{max} (ng/mL)	AUC (ng.day/mL)			T _{max} (day)	C _{max} (ng/mL)	AUC (ng.day/mL)		
				0-1d	0-14d	0-29d [†]			0-1d	0-14d	0-29d [‡]
1	SD	0.9	49.2	19.1	350	410	1.0	69.9	31.0	309	428
		1.0	17.6	5.2	96.2	70.1	1.1	16.4	7.8	68.5	43.1
2	Mean	1.7	55.2	16.9	344	392	2.3	60.3	23.4	320	318
	SD	0.7	10.4	5.0	56.2	42.9	0.8	27.5	5.5	121	70.6

[†] N = 6 [‡] N = 3

Histopathology Findings

Groups 1 & 2

Finding Sacrifice Day	Right Leg Dose Day 1	Group 1 Right Leg Dose - Day 1			Group 1 Left Leg Dose - Day 30			Group 2 Right Leg Dose - Days 1 and 30		
	Day 18	Day 44	Day 210	Day 301	Day 44	Day 210	Day 301	Day 44	Day 210	Day 301
Degeneration/ Regeneration	1, 2	2, 1, -	-, 1	1	2, 2, 3	-, -	-	1, 2, 1	-, 1	-
Fibroplasia	3, 4	-, -, -	-, -	-	3, -, 2	-, -	-	3, 3, 3	-, -	-
Fibrosis	-, -	2, 3, -	2, -	-	-, 2, -	-, 2	-	1, 2, -	-, 1	-
Infiltrate, Neutrophilic	1, 3	-, -, -	-, -	-	2, -, 2	-, -	-	-, 2, 1	-, -	-
Inflammation, chronic active	4, 5	-, -, -	-, -	-	4, 3, 5	-, -	-	3, 4, 4	-, -	-
Inflammation, granulomatous	-, -	1, 2, 1	3, -	-	-, -, -	1, 2	1	-, -, -	2, 4	-
Foreign Material, refractile	P, P	-, P, -	P, -	-	P, -, P	-, P	P	P, P, P	-, P	-
Edema	2, 2	-, -, 1	-, -	-	-, -, 1	-, -	-	-, 2, 1	-, -	-
Hemorrhage	-, -	-, -, -	-, -	-	-, -, -	-, -	-	-, 1, 1	-, -	-

Groups 3 & 4

Finding Sacrifice Day	Group 3 Right Leg Dose - Days 1 and 60		Group 4 Right Leg Dose - Days 1, 60 and 120	
	Day 74	Day 240	Day 134	Day 301
Degeneration/Regeneration	-, 2, 2, 1	-, -	1, 3, 1, 2	-, -
Fibroplasia	2, 2, 2, 3	-, -	2, 2, 2, 2	-, -
Fibrosis	-, -, -, 2	-, -	1, 2, 2, 4	2, 1*
Infiltrate, Neutrophilic	-, 2, 1, -	-, -	-, 2, -, -	-, -
Inflammation, chronic active	-, -, -, -	-, -	-, 5, -, 5	-, -
Inflammation, granulomatous	3, 4, 5, 4	1, 1	3, -, 3, -	3, 2*
Foreign Material, refractile	P, P, P, P	-, -	P, P, P, P	-, -
Edema	1, 2, -, 2	-, -	3, -, 3, -	-, -
Hemorrhage	-, -, 1, 1	-, -	-, 1, -, -	-, -

Severity Key: P = present; 1 = minimal; 2 = slight; 3 = moderate; 4 = moderately severe; 5 = severe
 *Note: For this table, microscopic findings for animal H38446 are presented based on the animal's treatment (Group 4, Day 301 sacrifice). Due to computer limitations, findings for this animal in all other areas of this report were recorded under the animal's original Group 1 designation (see footnote c in Methods text table).

Study title: Investigative Acute Local Tolerance Evaluation of Medisorb®

Naltrexone in Dogs Following Intramuscular Administration.

Key study findings:

- The intramuscular administration of Medisorb Naltrexone to dogs resulted in an injection site reaction (a localized swelling), similar to the reaction observed in the 6403-123 dog study.
- This study demonstrated that the severity of the reaction was dose-related and there were no differences between the lots of Medisorb Naltrexone evaluated.

Study no.:

Reference No: 6403-122;
Sponsor Reference No: AT-21-06

Volume # and page #: 1, 1-7

Conducting laboratory and location:

Date of Study Report: October 25, 2001

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity

Test Article Medisorb® Naltrexone, also identified as Medisort

Lot No. 192-2850A (nominal fill weight 481 mg; 34.4% drug load; same lot used in Alkermes dog study AT-21-05)

Lot No. 192-1750A (nominal fill weight 462 mg; 35.5% drug load; same lot used in Alkermes clinical study ALK21-001)

Lot No. 192-1800A (nominal fill weight 477 mg; 34.4% drug load; same lot used in Alkermes clinical study ALK21-001)

Test materials were supplied by Alkermes and stored in a freezer set to maintain 10 to -30°C.

Formulation/vehicle:

Vehicle: Medisorb® Diluent, also identified as Diluent [carboxymethylcellulose (CMC), polysorbate 20; and sodium chloride in water for injection]; Lot No. L02A9a (same lot used in Alkermes dog study AT-21-05) Lot No. F03A8a (same lot used in Alkermes clinical study ALK21-001)
The vehicle was supplied by Alkermes and stored at ambient conditions (15 to 30°C).

Control Material

Saline, 0.9% NaCl Injection; Lot No. J0L594.

The saline control was manufactured by and stored at ambient conditions (15 to 30°C).

Methods

Dose

Saline control was used as supplied. Medisorb Naltrexone was suspended with 1.2 mL of Medisorb Diluent just prior to administration (within approximately 5 to 10 minutes). The nominal microsphere concentration was approximately 280 mg/mL. For Group 3 (4 mL dose volume), three vials were pooled to deliver each microsphere dose. For Groups 1 and 2 (1 mL dose volume), one vial was used for each dose.

Four male and four female purebred beagle dogs approximately 7 to 10 months of age and 6.2 to 11.5 kg at study initiation, were utilized in the study administration.

The high dose (4 mL) was selected based on the equivalent single intramuscular dose in the AT-21-05 study. The low dose (1 mL) was selected to compare to the high dose.

Experimental Design:

Treatment Groups

Group	Number of Animals	Intramuscular Treatments		
		Left Side ^a	Right Side	Dose Volume
1	3	AT-21-05 dog materials (Medisorb Naltrexone lot 192-2850A and Medisorb Diluent lot L02A9a)	Saline	1 mL/site
2	3	ALK21-001 clinical materials (Medisorb Naltrexone lot 192-1750A or 192-1800A and Medisorb Diluent lot F03A8a)	Saline	1 mL/site
3	2	ALK21-001 clinical materials (Medisorb Naltrexone lot 192-1750A or 192-1800A and Medisorb Diluent lot F03A8a)	Saline	4 mL/site

^a The target doses were 280 and 1120 mg of Medisorb Naltrexone (98 and 392 mg naltrexone) for the 1 and 4 mL dose volumes, respectively.

Study design:

Prior to dose administration on Day 1, animals were lightly anesthetized by an intravenous injection of Domitor (sedative) and placed in lateral recumbency. The dosing sites were shaved and cleaned using a mild soap and each animal received injections of the test or saline control material into the left and right hind muscle (targeting biceps femoris), respectively. The dose volume was approximately 1 mL for Groups 1 and 2 and 4 mL for Group 3. The target dose for Groups 1 and 2 was 280 mg of Medisorb Naltrexone (98 mg naltrexone). The target dose for Group 3 was 1120 mg of Medisorb Naltrexone (392 mg naltrexone). The injection sites were uniquely identified with an indelible ink marker. After dosing, an intramuscular injection of Antisedan was given distant from the leg region as a reversing agent to the anesthetic. Each animal was monitored closely after dosing and returned to their cage once sternal

recumbency was regained. Each animal was observed twice daily for mortality and moribundity. Cageside clinical observations were made once daily and a detailed physical examination was performed once weekly. All animals were weighed on the first day of treatment (Day 1) and once weekly, thereafter. The injection sites were evaluated once daily.

Results:

No mortality occurred in this study. There were appreciable body weight changes during the study. Clinical effects were limited to injection site reactions. Following intramuscular injection of Medisorb Naltrexone, a clinically evident injection site reaction (a localized swelling) developed. This reaction was similar to that observed in the 6403-121 dog study. The injection site reactions were dose related in that the onset of the reaction was earlier, the size of the swelling was larger and the reaction was more persistent in animals that received 4 mL compared to those that received a 1 mL dose. Swelling was noted in both Group 3 animals, beginning on Days 2 or 4 and was observed to Day 15. Serosanguineous discharge was also present for one Group 3 animal on Day 10. Swelling was present in most animals in Groups 1 and 2 ranging between Days 7 and 15. Reddened skin was noted at a saline control injection site for one Group 3 animal on Day 7. No other findings were apparent for saline control injection sites.

At necropsy, residual test material was observed in most injection sites, either within the muscle and/or as a raised area in the adjacent subcutaneous region. Some injection sites exhibited a pale fluid filled pocket surrounding the test material within the muscle. In addition, tissue adhesion was present in the subcutaneous region between the skin and just above the muscle at the injection sites of some animals. Macroscopically, a greater amount of residual test material was observed for sites which received 4 mL compared to

Summary of Findings:

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Treatment – left leg	Animal #	Intramuscular Injection Sites – left leg
Group 1 AT-21-05 materials – 1 mL (Medisorb Naltrexone lot 192-2850A and Medisorb Diluent lot L02A9a)	H38498	SC adhesion, red, 4.0 x 4.0 cm
	H38502	Pale area at injection site, tan, 0.3 x 0.3 cm Pale fluid filled pocket surrounding test material within muscle
	H38503	Injection site swollen, tan raised area 0.4 x 0.4 cm Pale fluid filled pocket surrounding test material within muscle
Group 2 ALK21-001 materials – 1 mL (Medisorb Naltrexone lot 192-1750A or 192-1800A and Medisorb Diluent lot F03A8a)	H38499	Injection site swollen SC dark red area, 2.0 x 1.0 cm
	H38500	No remarkable findings
	H38504	Dark red raised area at injection site, 0.5 x 0.5 cm Test material present
Group 3 ALK21-001 materials – 4 mL (Medisorb Naltrexone lot 192-1750A or 192-1800A and Medisorb Diluent lot F03A8a)	H38501	Injection site swollen; dark red raised area 7.0 x 7.0 cm Pale fluid filled pocket surrounding test material within muscle SC adhesion, dark red, 7.0 x 7.0 cm
	H38505	Injection site swollen, tan raised area 3.0 x 2.0 cm Test material present Sore on skin involving the injection site, dark red, 0.4 x 0.4 cm SC adhesion, tan, 3.0 x 2.0 cm SC thickening, moderate

Study title: Investigative Local Tolerance Study of Medisorb® Naltrexone in Rabbits Following Intramuscular Administration

Key study findings:

- Intramuscular administrations of Medisorb Naltrexone and Medisorb Diluent to rabbits were well tolerated.
- There were no test material-related adverse effects apparent for clinical observations, body weight measurements and injection site observations.

Study no:

6403-123
 Sponsor Reference No: AT-21-07

Volume #, and page #: 1, 1-7
Conducting laboratory and location:
Date of Study Report: October 25, 2001
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity:

Test Article

Medisorb® Naltrexone, also identified as Medisorb

Lot No. 192-2850A (nominal fill weight 481 mg; 34.4% drug load; same lot used in Alkermes dog study AT-21-05) Lot No. 192-2350A (nominal fill weight 473 mg; 34.7% drug load; same lot used in Alkermes rabbit study AT-21-04) Test materials were supplied by Alkermes and stored in a freezer set to maintain -10 to -30°C.

Formulation/vehicle:

Vehicle Control: Medisorb® Diluent, also identified as Diluent [carboxymethylcellulose (CMC) polysorbate 20; and sodium chloride in water for injection]; Lot No. L02A9a (same lot used in Alkermes dog study AT-21-05) Lot No. F03A8a (same lot used in Alkermes rabbit study AT-21-04) The vehicle was supplied by Alkermes and stored at ambient conditions (15 to 30°C).

Methods

Dose

Medisorb Diluent (Group 1) was dosed as supplied. Medisorb Naltrexone (Groups 2 and 3) was suspended with 1.2 mL of Medisorb Diluent just prior to administration (within approximately 5 minutes). The nominal microsphere concentration was approximately 284 mg/mL and the target dose volume for each intramuscular injection was approximately 3.0 mL (a maximum intramuscular volume in rabbits). Two vials were pooled to deliver a single dose. The nominal dose per injection for Medisorb Naltrexone was approximately 852 mg (approximately 294 mg of naltrexone).

Three male and three female Hra:(NZW)SPF Rabbits approximately 18 to 20 weeks of age and 2,5 to 3.0 kg at study initiation, were utilized in the study. The rabbits were non-naïve and a sufficient washout period was provided prior to use on this study. All animals were healthy prior to test material administration. Animals were housed individually in suspended, stainless-steel cages.

Experimental Design:

Treatment Groups

Groups	Number of Animals ^a	Treatments			
		Dose Schedule	Intramuscular Injection ^a		
			Left Side	Right Side	Dose Volume
1	2	Day 1	Medisorb Diluent Lot L02A9a	Medisorb Diluent Lot L02A9a	3 mL/site
2	2	Day 1	Medisorb Naltrexone Lot 192-2850A ^b	Medisorb Naltrexone Lot 192-2850A ^b	3 mL/site
3	2	Days 1 and 8	Medisorb Naltrexone Lot 192-2350A ^c	Medisorb Naltrexone Lot 192-2350A ^c	3 mL/site/dose

- a Each animal received both intramuscular treatments.
- b Medisorb Diluent Lot L02A9a used for dose suspension of Medisorb Naltrexone, lot 192-2850A.
- c Medisorb Diluent Lot F03A8a used for dose suspension of Medisorb Naltrexone, lot 192-2350A.

Study design:

Prior to dose administration on Day 1, animals were lightly anesthetized by intramuscular injections of xylazine and ketamine. The dosing sites were shaved and cleaned using 70% isopropyl alcohol.

On Day 1, each animal received injections of the test or vehicle control material into the left and right hind muscle (targeting biceps femoris) at a dose volume of 3 mL/site. Animals in Group 3 received a second dose on Day 8 in close proximity to the Day 1 dosing site. Each intramuscular dose was delivered over approximately 5 to 10 seconds. The injection sites were uniquely identified with an indelible ink marker.

Intramuscular administration was used since this was the same dosing route evaluated in the 6403-119 dog study. The dose volume was selected as being the maximum single intramuscular dose volume (3 mL) deliverable in the thigh muscle of the rabbit. The repeat dose in Group 3 was included to assess the potential local reaction after two successive doses at or near the same site.

Each animal was observed twice daily for mortality and moribundity. Detailed clinical observations were made prior to dosing, approximately 1 hour post-dose, and once daily, thereafter. All animals were weighed on the first day of treatment (Day 1) and once weekly thereafter. The injection sites were visually evaluated at approximately 1 hour post-dose and once daily, thereafter.

All animals were sacrificed on Day 15 by an overdose of sodium pentobarbital followed by exsanguination. Animals were subjected to an abbreviated necropsy examination. Each injection site was observed for any macroscopic changes, photographed and preserved in 10% formalin for future storage.

Results:

All animals were clinically normal throughout the study and survived to the scheduled necropsy. There were no adverse effects on body weights during the study. Intramuscular injection of Medisorb Naltrexone or Medisorb Diluent did not result in any adverse reactions at the local injection sites. A slight raised area was noted one hour after dosing for one animal each from Group 2 on Day 1 and Group 3 on Day 8. The raised areas in both animals did not persist and injection sites were clinically normal one day after dosing. No other test or vehicle control related injection site findings were noted clinically.

The macroscopic findings were limited to the intramuscular injection sites. At necropsy, a light focus interpreted as residual test materials was observed at all intramuscular Medisorb Naltrexone dose sites for Groups 2 and 3. Findings for all Group 2 injection sites consisted of a single, firm and tan area within the muscle. For all sites in Group 3, one or two distinct firm tan areas were observed within the muscle. One Group 3 site

also showed a small light focus area of residual material on the surface of the muscle. There were no macroscopic findings for any of the vehicle control treated sites (Group 1). There were no other macroscopic findings reported.

Summary of Findings:

Treatments	Animals	Gross Necropsy Observations	
		Left Injection Site	Right Injection Site
Medisorb Diluent Lot L02A9a	F06460M	No findings	No findings
	F06471F	No findings	No findings
Medisorb Naltrexone Lot 192-2850A	F06455M	Light focus, 8.0 x 1.5 cm	Light focus, 5.0 x 2.0 cm
	F06487F	Light focus, 5.0 x 2.5 cm	Light focus, 5.5 x 2.5 cm
Medisorb Naltrexone Lot 192-2350A	F06457M	Light focus, 2 areas (9.0 x 1.0 cm and 5.5 x 1.5 cm)	Light focus, 10.0 x 2.5 cm
	F06486F	Light focus, 2 areas (6.0 x 2.5 cm and 6.0 x 2.5 cm)	Light focus, 11.0 x 2.5 cm; Light focus on muscle surface, 1.5 x 0.5 cm

2.6.6.8 Special toxicology studies: No special toxicology studies were submitted,

Impurities

Drug Substance

Status of Impurity Issues for Naltrexone Base Anhydrous

The potential impurity in Naltrexone is
The structure of this impurity is depicted below.



The briefing document of February 2, 2005 Pre-NDA meeting contains the following information concerning the level of impurity in lots of the drug substance: It is stated in the package that "historical lots have been analyzed and found to contain between of the impurity using an unvalidated research method. This impurity is a process impurity in the API and not a degradation product.

The February 2, 2005 Pre-NDA briefing document also indicates that plans to amend their DMF in March 2007 to include a revised process, test method and specification to demonstrate control of the subject impurity under the limit.

At the February 2, 2005 meeting the timeline for establishing control over the level of impurity in naltrexone base anhydrous drug substance was reconfirmed. The level of impurity will be controlled in the drug substance by the supplier.

It was decided to establish an interim release specification of , which will then be followed by a final release specification of μm after March 2007.

At the February 2, 2005 meeting the agency indicated that the timing of the approval of the Vivitrex NDA will not be affected by the March 2007 timing for _____ to implement the final _____ specifications.

It is indicated in the NDA submission that *Alkermes* is committed to establish a specification for _____ impurities in the drug substance naltrexone base anhydrous once changes were made to _____ DMF _____

Drug Product Impurities

Three naltrexone related impurities _____ and _____ have been determined to be present in Vivitrex® drug product at levels greater than or equal to the ICH Q3B(R) reporting threshold of 0.1% (assuming a 380 mg daily dose). _____ are drug product manufacturing process impurities and degradation products. _____ is a drug substance impurity carried through the drug product manufacturing process from the drug substance.

The impurity qualification threshold of _____ in the Vivitrex drug product will be applied to all drug product impurities and degradation products. No Vivitrex drug product impurity has been found to exceed _____ either through drug product manufacturing or over the course of stability studies, therefore no further qualification is required.

_____ is a drug substance impurity, a Vivitrex drug product process impurity and degradation product. _____ is present in the naltrexone drug substance _____

_____ has been identified in the Vivitrex drug product _____

_____ will be controlled in the drug substance with a release specification of _____ and in the drug product with a release specification of _____. Also, _____ in the Vivitrex drug product on stability will be controlled with a shelf life criterion of _____

_____ is a drug substance impurity. The level of _____ does not increase during the microsphere manufacturing process or over the course of stability studies. _____ has been identified in the Vivitrex drug product _____

formation of _____ during the manufacturing process or on stability would require _____ this is believed to not be possible during the manufacturing process or on stability of Vivitrex microspheres. _____ is considered a drug substance impurity only _____ will be controlled in drug substance with an interim release specification of \leq _____ and after March 2007 with a release specification of _____. This strategy is documented in the information package dated, December 10, 2004 (IND No. 61,138, Serial No. 069) and discussed with the Agency, February 2, 2005. As _____ is controlled in the drug substance, no drug product release or shelf life criterion is required.

Enantiomeric Impurity:

Other Possible Known Impurities:

Several other impurities are known to be possible in the Vivitrex drug product. These impurities have been listed by Alkermes' drug substance supplier. They include _____

Each of these impurities is controlled in the drug substance with a specification of _____. In addition, these impurities are controlled in the Vivitrex drug product with the specification of any other impurity \leq 0.1%.

Unknown Impurities:

Unknown impurities should be controlled in the Vivitrex drug product with the specification of any other impurity \leq 0.1%. Any impurity $>$ 0.1% will be identified according to ICH Q3B(R).

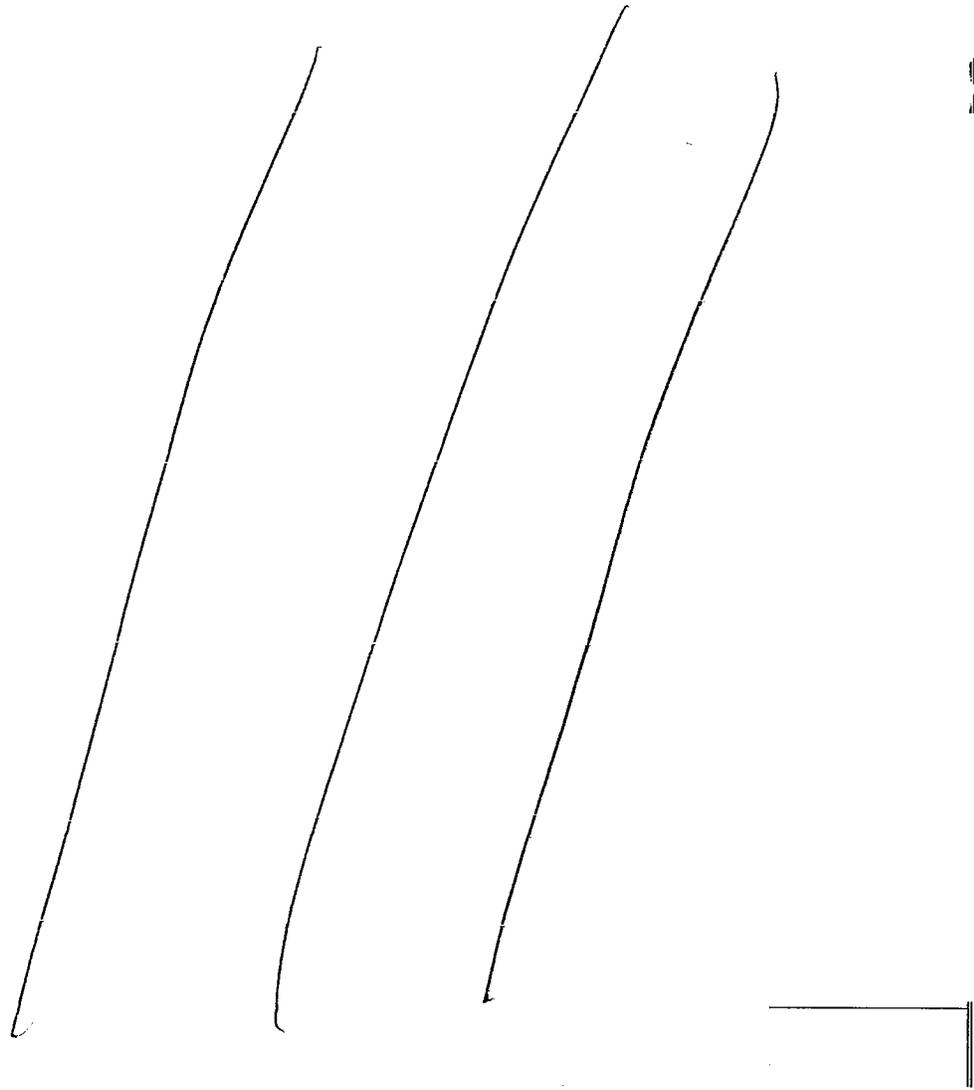
Residual Solvents:

_____ are used in the manufacturing process for Vivitrex. _____ will be controlled with a specification of _____ will be controlled with a specification of _____. Each of these solvent control levels is _____

Alkermes has evaluated [redacted] content in Vivitrex microspheres. The Vivitrex microsphere manufacturing process was designed to minimize the amount of residual [redacted] in the final product.

[redacted] Residual [redacted] was determined for several batches. A mean value of 5 batches of [redacted] with a maximum of [redacted] was determined. The expected level of human exposure of [redacted] in the anticipated dose of Vivitrex microspheres is approximately [redacted] dose (based on a value of [redacted] of approximately [redacted] microspheres delivering 380 mg naltrexone). This exposure is several orders of magnitude less than that associated with systemic effects in animals and is not expected to present any issues regarding toxicity. Due to the low level of [redacted] determined in the Vivitrex microspheres and the lack of toxicity concerns at these levels, Alkermes proposes no test or specification provision [redacted]

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2.6.6.9 Discussion and Conclusions

The Vivitrex nonclinical safety studies completed by the sponsor included programs of general toxicity and local tolerance testing. Reference is made to the agency's finding of safety (NDA 18-932 submitted by Dupont and Endo Laboratories for oral formulations of naltrexone), under the provisions of section 505(b)(2) of the United States Federal Food, Drug, and Cosmetic Act. Additionally, toxicity data from scientific publications are also considered for reviewing the safety of this product.

The proposed clinical dose of Vivitrex microspheres is predicted to produce a mean naltrexone exposure approximately 3.9-times the aggregate exposure generated by 28 days of the standard oral naltrexone dose of 50 mg/day. Despite this exposure increment, the doses in all of the naltrexone nonclinical studies (NDA 18-932) are calculated to exceed those resulting from the maximum proposed dose of Vivitrex microspheres.

In the 3-month, 3-cycle SC repeated-dose toxicokinetic safety study in monkeys doses of 20, 50 and 75 mg naltrexone/kg (57, 143, 215 mg microspheres/kg) were administered in dose volumes < 1.5 mL/ injection. Drug exposure was limited by the availability of suitable SC sites, since up to 6 injection sites were required in high-dose animals to administer a full dose. No evidence of systemic toxicity was observed in the study at any dose, but as with the single dose monkey study, local injection site effects were observed. Toxicokinetic analysis revealed a dose-related increase in naltrexone AUCs.

Comparison of Human and Monkey naltrexone concentrations (Mean ± SD)

STUDY	AT-21-02				AT-21-03			ALK21-005	
STUDY DESIGN	1-MO TOX (SINGLE DOSE) MONKEY				3-MO TOX (DOSE Q28 DAYS X3) MONKEY			CLINICAL SAFETY STUDY	
NTX Dose (mg/kg: nonclin) (mg: clin)	20	50	200	50	20	50	75	380	1400 mg 50 mg/day
Route	SC	SC	SC	IM	SC	SC	SC	IM	Oral
N	4	4	6	4	6	6	10	10	14
C _{max} (ng/mL)									
Cycle 1	31.8 (8.4)	64.3 (6.4)	265 (25)	106 (30)	89.9 (107)	102 (75)	72.1 (36.6)	28.0 (12.2)	13.7 (10.6)
Cycle 2	-	-	-	-	38.9 (25.7)	72.6 (34.5)	214 (226)		
Cycle 3	-	-	-	-	27.8 (5.3)	82.5 (60.5)	123 (50.5)		
AUC _{0-28d} (ng·day/mL)									
Cycle 1	187 (45.7)	449 (155)	2115 (654)	609 (20.5)	423 (389)	588 (277)	513 (130)	160 (24.2)	41.1 [‡]
Cycle 2	-	-	-	-	307 (125)	556 (260)	1567 (1323)		

Cycle 3	-	-	-	-	164 (38) [†]	447 (127) [†]	751 (282) [†]		
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NTX = naltrexone

* AUC_{0-28d}

† AUC_{0-34d}

‡ AUC_{28d} was calculated as the mean of AUC_{1d} * 28

Human exposure to naltrexone in Vivitrex at the proposed dose of 380 mg/month (7.6 mg/kg) produced naltrexone exposures estimated by interpolation at approximately 388% of the aggregate exposure from 28 days of oral dosing with the 50 mg tablet (AUC_{0-28d} 41.2 ng-day/mL for oral and 160 ± 24.2 ng-day/mL for a 380 mg dose of Vivitrex microspheres. Similarly, toxicology studies conducted with Vivitrex suspension also exceeded the clinical exposure level.

The monkey is the only nonclinical species to produce 6β-naltrexol in amounts sufficient to quantitate. Only minimal 6β-naltrexol is produced in rabbits. In nonclinical testing, adequate dose multiples were utilized in rhesus monkeys to produce a 6β-naltrexol AUC_{0-29d} of approximately 43% that produced with the proposed clinical dose of naltrexone in humans. The opioid receptor antagonist activity of 6β-naltrexone is less than that of naltrexone by a factor of about 150 (based on hot plate test). However, despite its lower potency, 6β-naltrexol may enhance the duration of opiate antagonism due to its longer half-life. The enzyme that is involved in the generation of 6β-naltrexol is dihydrodiol dehydrogenase (DD4), which is a cytosolic enzyme, found primarily in the liver of humans, but has been reported to be most concentrated in the kidney of the monkey.

The local tolerability studies in rabbits and the single dose and multiple dose monkey studies had substantially similar outcomes for injection site evaluations. The multiple doses IM local tolerability study conducted in the dog was significantly different in design and outcome and is described separately.

Exposure Multiples for Vivitrex (naltrexone long-acting injection) Nonclinical Toxicology Studies

SPECIES	STUDY TYPE	STUDY NUMBER	DOSE MULTIPLE RELATIVE TO VIVITREX 380 MG		
			MG/KG BASIS*	BODY SURFACE AREA BASIS	AUC BASIS
Monkey	Single-dose toxicity	AT-21-02	26	8.7	17.5 [†]
Monkey	Multiple-dose toxicity	AT-21-03	10	3.3	4.7 [‡]

* Based on a 50 kg human
[†] AUC_{0-29d} Monkey, AUC_{0-28d} Human
[‡] AUC_{0-33d} Monkey, AUC_{0-28d} Human

SC and IM routes of administration were used in the rabbit and monkey single dose toxicity studies to aid in selecting a clinical route of administration. The SC route was used for the repeated dose monkey study because insufficient IM sites were available for

depot injection in that species. Both SC and IM injections of Vivitrex microspheres and Medisorb Placebo microspheres in monkeys and rabbits resulted in a clinically visible injection site enlargement attributable to the mass of test material and pathological evidence of a foreign body reaction at the depot site.

Comparison of Human and Monkey 6β-naltrexol concentrations (Mean ± SD)

STUDY	AT-21-02				AT-21-03			ALK21-005	
STUDY DESIGN	1-MO TOX (SINGLE DOSE) MONKEY				3-MO TOX (DOSE Q28 DAYS X3) MONKEY			CLINICAL SAFETY STUDY	
NTX Dose (mg/kg: nonclin) (mg: clin)	20	50	200	50	20	50	75	380	1400 mg (50 mg/day)
Route	SC	SC	SC	IM	SC	SC	SC	IM	Oral
N	4	4	6	4	6	6	10	10	14
C _{max} (ng/mL)									
Cycle 1	1.6 (0.8)	4.9 (1.1)	12.3 (2.0)	4.0 (1.6)	2.7 (1.8)	5.1 (2.2)	6.1 (2.2)	34.2 (12.9)	138.7 (36)
Cycle 2	-	-	-	-	2.9 (0.9)	9.6 (8.5)	13.1 (7.3)		
Cycle 3	-	-	-	-	4.3 (2.9)	5.9 (2.3)	10.5 (4.0)		
AUC _{0-28d} (ng·day/mL)									
Cycle 1	11.1 (5.6)*	40.6 (12.5)*	127 (22.7)*	35.0 (12.5)*	20.1 (7.3)	46.8 (13.7)	67.7 (22.1)	294 (70.4)	1002 [‡]
Cycle 2	-	-	-	-	20.7 (7.8)	58.5 (31.3)	103 (32.3)		
Cycle 3	-	-	-	-	26.0 (12.4) [†]	58.2 (20.6) [†]	97.6 (28.5) [†]		

NTX = naltrexone

* AUC_{0-28d}

† AUC_{0-34d}

‡ AUC_{28d} was calculated as the mean of AUC_{1d} * 28

In the intramuscular and subcutaneous 8-month local tolerance study in rabbits, treatment related effects were found at the injection sites. Rabbits that received 195 mg naltrexone in 562 mg microspheres dosed SC in a 2 mL dose volume exhibited a local enlargement at the injection sites attributed to the presence of the test material depot. The intramuscular dose sites of Vivitrex suspension in rabbits (293 mg naltrexone; 843 mg microspheres; 3 mL dose volume) were clinically normal. Necropsies conducted at 8 and

30 days after dosing revealed that IM and SC sites consisted mainly of residual test material appearing as white to tan colored material. Clinically and by gross pathological examination, it was evident that the test materials were gradually resorbed as the study progressed. Microscopically the polymer microspheres were progressively degraded and the accompanying foreign-body response and fibrosis at the IM and SC injection sites also gradually diminished. These results confirm the observations made in the repeated dose monkey study where granulomatous inflammation consisting of macrophages and multinucleated giant cells along with fibrosis were observed at injection sites in the three month sacrifice and were subsequently seen to be resolving by the 6 month sacrifice. By 6 months after dosing in the monkey and 8 months after dosing in rabbits, residual polymer was either no longer present, or only present in small amounts. Additionally, the histological changes related to the foreign body response were returning to normal. In the rabbit study, where IM and SC routes were both evaluated, local microsphere reactions appeared to resolve more rapidly at the IM sites.

A 10-month local tolerance study was completed in dogs (AT-21-05). No systemic toxicity was apparent after administration of Vivitrex suspension in this study. The objective of this study was to accurately mimic human dosing conditions (i.e., multiple dosing at the same or closely spaced sites while utilizing a dose and administered volume comparable to that proposed for human dosing). Vivitrex microspheres (394 mg naltrexone; 1144 mg microspheres; 4 mL dose volume) was administered on day 1 in all groups, followed by further doses on day 30 in groups 1 and 2, day 60 in group 3, and days 60 and 120 in group 4. All animals received active formulation. The study also included a treatment arm in which dogs were dosed in the same location at an interval of 30 days, rather than the recommended 60 day interval when gluteal injections are alternately administered on left and right sides. No systemic toxicity was apparent after administration of Vivitrex suspension on any occasion during the study. A prominent injection site reaction occurred in all animals. The local reaction consisted of skin swelling approximately 1 to 5 cm in diameter. An area of discoloration up to 7 cm in diameter was also noted at the injection sites of some animals. Residual test material within the muscle and in some cases on the proximal muscle surface was observed at all necropsy intervals and at all injection sites. Adhesions between the skin and skeletal muscle were also observed grossly at the injection sites in some animals sacrificed 2 weeks after dosing.

Histopathological findings at injection sites consisted predominantly of an inflammatory reaction with fibrosis. At sites evaluated approximately 2 weeks after dosing, a chronic active inflammatory reaction (moderate to severe) was observed that was characterized mainly by macrophages and multinucleated giant cells with smaller numbers of neutrophils, eosinophils and lymphoplasmacytic cells. Granulomatous inflammation (minimal to moderately severe) was the primary histopathological change observed at the Day 210 sacrifice (6 to 7 months post- dose). The inflammatory response and accompanying fibrosis diminished significantly between the 2-week post-dose sacrifices and the Day 210 sacrifice.

The injection site reactions in dogs were apparent soon after administering the first dose and were most severe between 1 to 3 weeks post-dose. Gross and microscopic examinations both indicated that injection site reactions were not enhanced when three successive 4 mL injections of Vivitrex suspension were administered at monthly intervals in very close proximity to each other. Additionally, there was also no effect on pharmacokinetic parameters after multiple dosing. All reactions gradually diminished and the sites were essentially normal in appearance upon gross examination by the end of the study period.

The proposed clinical Vivitrex dose (380 mg) produced one month human naltrexone exposure levels approximately 3.9-times that observed with one month of oral dosing at 50 mg of naltrexone/day (The 3.9 factor is based on the AUC_{0-28d} for Vivitrex suspension versus the $AUC_{1d} * 28$ days for oral naltrexone). Naltrexone exposure in animal studies supporting the NDA for the oral formulation exceeded the proposed Vivitrex human naltrexone exposure based on mg/kg and BSA comparisons.

Comparison of NOAELs from Oral Naltrexone Studies and Dose Multiples Relative to Vivitrex suspension

SPECIES	TYPE OF STUDY	NOAEL* (mg/kg/day)	ORAL DOSE MULTIPLE†	DOSE MULTIPLE RELATIVE TO VIVITREX 380 mg‡	
				mg/kg BASIS	BSA BASIS
Monkey	Chronic	20	20	5.1	1.7
Rat	Subchronic	70	70	18	3.0
Dog	Subchronic	40	40	10	5.0
Rat	Reproductive	30	30	7.7	1.3
Rat	Teratology	200	200	51	8.5
Rabbit	Teratology	60	60	15.4	5.1
Mouse	Carcinogen'ity	100	100	26	2.1
Rat	Carcinogen'ity	100	100	26	4.3

* NOAEL: No Observed Adverse Effect Level

† Calculated using a therapeutic human dose of 1 mg naltrexone/kg/day (derived for the standard daily oral naltrexone dose of 50 mg, and an assumed body weight of 50 kg).

‡ Calculated by comparison of the AUC_{0-28d} for Vivitrex to the aggregate $AUC_{1d} * 28$ days for oral formulation (AUC_{0-28d} 41.2 ng·day/mL for oral and 160 ± 24 ng·day/mL for a 380 mg dose of Vivitrex resulting in a conversion factor of 3.9)

^{||} mg/kg to BSA conversion factors adapted from Freireich 1966 (20) and Casarett and Doull's Toxicology (21).

Naltrexone increased the incidence of early fetal loss when given to rats or rabbits. Findings of an increased incidence of pseudopregnancy and decreased pregnancy rates

were seen in rats at 100 mg/kg (13-times the recommended monthly human dose of Vivitrex microspheres on a mg/kg basis). Other observations included reports of increased resorptions, increased incidence of stillborn pups, and lower body weights of pups. However, no epidemiological evidence of developmental and reproductive toxicities attributable to naltrexone has emerged after nearly 20 years of clinical use. Further evidence of the clinical reproductive safety of naltrexone is provided by the finding that use of naltrexone in the management of pregnant heroin users has not resulted in any identifiable effects of naltrexone on the mother or fetus.

Genetic toxicity testing of naltrexone resulted in no positive findings except in a few minor assays. Negative genotoxicity studies (covering the ICH standard battery of tests) included the *Salmonella* and *E. coli* microbial gene mutation assays, the mouse micronucleus assay, the heritable translocation assay, CHO cell sister chromatid exchange assay, and the mouse lymphoma gene mutation assay. The following minor assays gave positive results: *Drosophila* recessive lethal frequency assay, non-specific DNA damage in repair tests with *Escherichia coli* and WI-38 cells, and urinalysis for methylated histidine residues. The significance of these findings is unknown.

In rat and mouse carcinogenicity studies 30 and 100 mg/kg/day of naltrexone in the diet for 2 years was not found to be carcinogenic. At the doses evaluated growth rates were slightly depressed in a dose related manner in both species and food consumption was reduced in mice (Rosenkrantz, 1984). The frequency and location of predominant tumors in both species were similar in treated and untreated groups with the exception of a small increase in the incidence of vascular tumors and mesotheliomas in rats receiving naltrexone. Trend analysis adjusted for mortality revealed a significant positive dose-related increase in the incidence of vascular origin tumors for both males and females. However, this positive relationship was due to a small increase in tumors for males and females in the high dose group only. When the high dose rates were compared with those of matched controls by Fisher Exact Test, there were no significant differences. The trend analysis also showed a weak positive dose response relationship for mesothelioma in males. These tumors were generally late occurring and were not thought to contribute significantly to the overall mortality of treated groups.

The medical use of biodegradable polymers, such as the polylactide-co-glycolide (PLG) polymer of Vivitrex microspheres, is well established. Polyesters of the alpha-hydroxy carboxylic acids, including copolymers of lactide and glycolide, and homo-polymers containing poly-L-lactide, poly-D-lactide or polyglycolide are among the most widely used, having been successfully employed in medical devices such as sutures (Dexone and Vicryle) and in a variety of forms for soft tissue and bone fixation (meshes, screws, pins, nails, plates and splints). PLG polymers have been used in sustained delivery of proteins and peptides (Nutropin Depot, Zoladex, Luprone Depot, De-Capeptyle SR and Sandostatin LAR Depot). The wide use of the polymers is based in part on evidence of good tissue tolerance and unimpaired tissue regeneration in animals and humans.

Degradation of PLG depends largely on hydrolysis of ester linkages, the rate of which is influenced by the physical and chemical characteristics of the polymer and is also

dependent in part on cellular and enzymatic effects.

The degradation products are the



Polymer degradation products are generated slowly over time as the microspheres degrade, and this slow rate of formation and release contributes to their low toxicity. Animal safety studies performed by the Sponsor showed no acute, subacute, or chronic systemic toxicity is anticipated at the doses of PLG administered in Vivitrex microspheres. PLG produces local inflammation at the site of injection, but this is reversible.

Local tolerance studies with PLG microspheres completed by the Sponsor in rabbits found a non-irritating inflammation at injection sites consisting of macrophage infiltration and diffuse intralesional fibrosis. PLG microspheres remained localized at the subcutaneous injection site. By about 5 months after dosing, PLG microspheres (approximately 150 mg subcutaneous dose) were fully degraded and the inflammatory tissue reaction was resolved at the local subcutaneous injection site.

A review of relevant literature indicated that PLG polymer or its metabolites do not cause reproductive and developmental effects in animal studies at the doses expected to result from PLG-polymer dosing. Subcutaneous implantation of suture material composed of PLG (Vicryl) was not teratogenic in rats and rabbits at 1000 mg/kg. Moreover, a search of the literature on clinical usage of various approved controlled release drug formulations that utilize PLG polymers did not identify any reports of adverse reproductive and developmental effects. No reproductive toxicity studies with Naltrexone along with the PLG polymer were done. Negative results have been reported for PLG in the Ames assay. No clastogenicity study with PLG polymer was found in the available literature. In a carcinogenicity study, intraperitoneal implants of Vicryl (50 mg/kg) for up to 2 years in rats did not cause any treatment-related tumors. The composition of the PLG component in this study was not given. No carcinogenicity study with the PLG along with Naltrexone has been done.

Microsphere Diluent is used to suspend Naltrexone microsphere products for injection. Microsphere Diluent is a solution that consists of carboxymethylcellulose sodium salt, (CMC); polysorbate 20 (Tween 20) and sodium chloride USP in Water for Injection (WFI). USP grade sodium chloride is considered non-toxic. Carboxymethylcellulose sodium salt is generally recognized as safe (GRAS) and is included in many currently marketed drugs and cosmetics with no signs of toxicity. Tween 20 is also widely used in cosmetics, foods and pharmaceutical preparations in customary use and is regarded as nontoxic and nonirritant at the concentration in Microsphere Diluent. Studies of Microsphere Diluent conducted by Alkermes include AT-03-09, AT-21-01 and AT-21-04, the latter two of which contained vehicle control

groups, have found Microsphere Diluent to be well tolerated. Slight local redness and enlargement was noted upon administration of Microsphere Diluent to rabbits. Other than a weak inflammatory cell infiltration, no significant clinical, macroscopic, or microscopic findings were noted at SC injection sites 8 days post-administration.

2.6.6.10 Tables and Figures

Nonclinical Safety Studies Sponsored by Alkermes

STUDY TYPE AND DURATION (ALKERMES REFERENCE NUMBER)	ROUTE OF ADMINISTRATION	SPECIES	COMPOUND ADMINISTERED
Single-dose toxicity (AT-21-02)	SC and IM	Monkey	Vivitrex suspension
Repeat-dose toxicity – 3-month with 3-month recovery (AT-21-03)	SC	Monkey	Vivitrex suspension
Local Tolerance – 1-month (AT-21-01)	SC and IM	Rabbit	Vivitrex suspension
Local Tolerance – 8-month (AT-21-04)	SC and IM	Rabbit	Vivitrex suspension
Local Tolerance – 10-month (AT-21-05)	IM	Dog	Vivitrex suspension
Local Tolerance – 2 week (AT-21-07)	IM	Rabbit	Vivitrex suspension
Local Tolerance – 2 week (AT-21-06)	IM	Dog	Vivitrex suspension
Local Tolerance – 8 days (AT-07-01)	SC	Rabbit	PLG microspheres
Local Tolerance – 365 days (AT-07-02)	SC	Rat	PLG microspheres and discs
Local Tolerance – 8 days (AT-03-09)	SC	Rabbit	Microsphere Diluent

*Non-GLP studies.

2.6.7 TOXICOLOGY TABULATED SUMMARY: NA

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Unresolved toxicology issues (if any): In vitro release data for this compound submitted by the sponsor as well as from the published literature show drug content strongly affects naltrexone microsphere drug release behavior. ~~_____~~

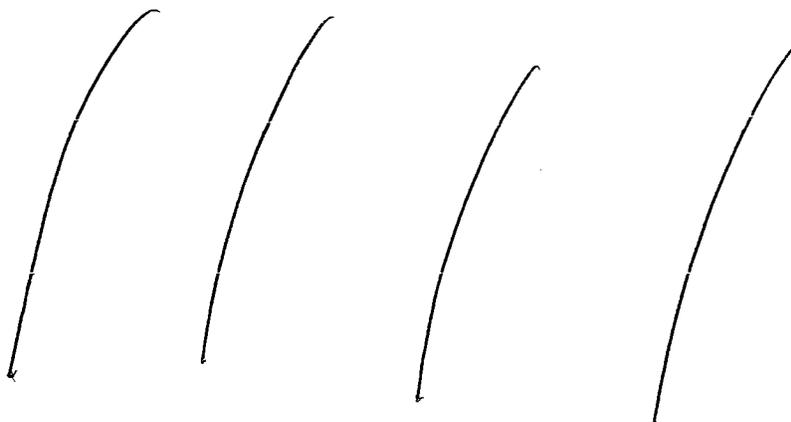
So, it can be concluded that the biodegradation should be unique to this polymer formulation and extrapolation of data from other products could be inaccurate. Local tissue reaction due to presence of the polymer greatly exceeds the therapeutic release of drug substance leading to accumulation of bioburden. A single injection of the product in rat and monkey requires 6-8 months after dosing to get rid of the residual polymer and complete reversal of the local histological changes. In the dog study the recovery sacrifices conducted 6 months after the last dosing and up to 10 months after the first dosing (Group 1), demonstrated that polymer degradation was progressive and that it was accompanied by evidence of reversal of local inflammation and fibrosis. Safety issues due to prolonged tissue inflammation, infection includes

- 1) Polymer degradation characterization does not appear complete.
- 2) Due to increase in AUC with this product, reproduction studies based on total daily exposure based on body mass do not appear to be relevant.
- 3) Similar interpretation can be drawn for the carcinogenicity study.

Recommendations:

It is recommended that the reproductive toxicity studies and the carcinogenicity study be performed with this compound in current clinical formulation to accurately evaluate the safety of the compound and for labeling purposes. Although the polymer degradation characterization is not complete, since progressive recovery was observed repeat dose toxicity study with the recovery phase need not be done at this point.

Suggested labeling:



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this page is the manifestation of the electronic signature.**

/s/

Mamata De
12/16/2005 04:39:01 PM
PHARMACOLOGIST

R. Daniel Mellon
12/16/2005 04:43:33 PM
PHARMACOLOGIST

I concur with Dr. De's recommendation that from the
nonclinical perspective the NDA is considered not approvable
at this time and with her recommendations for
nonclinical studies.