# 3.4.6 Local tolerance

**Table 2.6.7.16a. Local Tolerance (In Vitro)**
**Test Article: Cinacalcet Hydrochloride (AMG 073)**

| Species/ Strain | Method of Administration | Doses | Gender and No. Per Group | Noteworthy Findings | Study Number |
|---|---|---|---|---|---|
| Anticoagulated rat, non-human primate, and human blood | In vitro direct contact | 5 mg/mL AMG 073 Mesylate (50mM lactate, 190mM dextrose, pH 4.20); 1.33 mg/mL AMG 073 HCl (50mM acetate, 222mM dextrose, pH 4.25); 5 mg/mL AMG 073 Mesylate (50mM acetate, 197mM dextrose, pH 4.20); 5 mg/mL AMG 073 Mesylate (50mM acetate, 4% w/v ethanol, pH 4.27) | n/a | All test articles and vehicle controls induced hemolysis in rat, non-human primate, and human blood. | 100359 |
| Human blood | In vitro direct contact | AMG 073 Mesylate and AMG 073 HCl at 0.005, 0.05, 0.5, 5.0, 50, 500, and 1000 µg/mL | n/a | Hemolysis observed in all test groups with a general dose-response trend | 100399 |
| Human blood | In vitro direct contact | AMG 073 in the following vehicles: Intralipid-low shear; Intralipid-high shear; 10mM Acetate/278mM Dextrose; 10mM Acetate/275mM Mannitol; 5% Captisol/50mM Phosphate; 11.6% Captisol/NaOH to adjust (pH~7.85); HCl to adjust pH(~4.10); 0.5% Tween 80/50mM Phosphate; DMSO, 15% Captisol/10mM Phosphate, pH7.0 All at 1 mg/mL concentration and at 5, 50, or 500µL volume | n/a | Inconclusive results for Intralipid samples due to opaque white appearance interfering with optical density. The following formulations were negative for producing hemolysis: 11.6% Captisol/NaOH to adjust and 15% Captisol/10mM Phosphate,pH7.0. All remaining test article formulations were positive for producing hemolysis in human blood at 1 mg/mL. Increasing hemolytic index values occurred with higher volumes of formulations used in the assays | 100482 |

**Table 2.6.7.16b. Local Tolerance (In Vivo)**
**Test Article: Cinacalcet Hydrochloride (AMG 073)**

| Species/ Strain | Method of Administration | Doses | Gender and No. Per Group | Noteworthy Findings | Study Number |
|---|---|---|---|---|---|
| New Zealand White Rabbit | Topical | 0.5g/ 1mL distilled $H_2O$ | 3 males | AMG 073 caused only a very slight erythema reaction in 1/3 animals at the 4-hr observation point. No other dermal irritation was observed | 100325 |
| New Zealand White Rabbit | Instilled into the eye | 32 mg | 3 females | AMG 073 caused corneal opacity, iridal irritation, and severe conjunctival irritation in all 3 animals. Corneal opacity (3/3 animals) and conjunctival irritation (1/3 animals) were still present at day 21 after treatment | 100324 |
| Guinea Pig | Topical, Intradermal | Intradermal 0.1 mL/site (0.5% w/v)<br>Topical 0.3 mL/site (25% w/v)<br>Challenge 0.2 mL/site (25% w/v) | 10 M & F (test article)<br>5 M & F (vehicle control)<br>3 M & F (positive control) | Mild Sensitizer (Grade II) | 102943 |

Local tolerance studies showed that cinacalcet is a severe eye irritant and a mild dermal contact irritant. In the guinea pig sensitization study, cinacalcet was a mild dermal sensitizer.

4 page(s) have been removed because it contains trade secret and/or confidential information that is not disclosable.

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## 3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Pending agreement on the label, this NDA can be approved (AP)

Recommendations: Based on *in vitro* and *in vivo* nonclinical data, thorough evaluation of clinical trial data for any events related to cardiac conduction abnormalies under resting or stress conditions (EKG), myocardial and coronary artery disease, and CNS excitation (seizures) is recommended.

Suggested labeling: Labeling changes for the "PRECAUTIONS" section relating to carcinogenicity and reproductive study results are appended (Team Leader Memo, K. Davis-Bruno, February 10, 2004)

Signatures (optional):

Reviewer Signature /S/

Supervisor Signature /S/ Concurrence Yes ___ No ___

## 3.7 APPENDIX/ATTACHMENTS

- INITIAL IND REVIEW
- ECAC MEETING MINUTES
- PROPOSED LABEL
- SUGGESTED LABELING CHANGES (Team Leader Memo, K Davis-Bruno, February 10, 2004)

**IND 56,010**

Reviewer: Gemma Kuijpers
Review Date: June 19, 1998

## PHARMACOLOGY REVIEW OF ORIGINAL IND

Sponsor: Amgen Inc.
Submission: May 21, 1998
Serial Nr.: 000
Drug: AMG 073
Category: Calcimimetic
Indication: Treatment of secondary hyperparathyroidism

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Gemma A. Kuijpers, Ph.D.

cc: IND Arch
HFD-510
HFD-510/Steigerwalt/Kuijpers/Lutwak/Hedin
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**IND 56,010**

| | |
|---|---|
| Sponsor: | Amgen Inc. |
| Drug: | AMG 073<br>(N-[1-(R)-(1-naphthyl)ethyl]-3-[3-(trifluoromethyl)phenyl]-1-aminopropane |
| Category: | Calcimimetic |
| Indication: | Treatment of secondary hyperparathyroidism (HPT) |
| Dosage formulation: | |
| Dosage route: | Oral |
| Clinical Status: | Phase I/II |
| Memos: | Pre-IND meeting: May 6, 1998 |
| Related IND's: | |

## INTRODUCTION

Secondary hyperparathyroidism (HPT) is characterized by parathyroid gland hyperplasia and eleveated circulating PTH levels. The disease is a consequence of chronic renal failure (CRF) and/or end stage renal disease (ESRD). Prevalence of CRF is 250,000 in US. The bone of HPT patients has a fibrotic marrow space, and there is a predisposition to fractures and impaired erythropoiesis. Excess PTH may also affect various other tissues (heart, neurons, muscle, vessels). Hypercalcemia can develop in severe cases. Together with hyperphosphatemia this can lead to soft tissue calcification. Primary HPT is associated with persistently elevated serum calcium and PTH levels. Most cases are dye to parathyroid gland adenoma. It is frequently asymptomatic, but can lead to high bone turnover, osteolysis, marrow fibrosis and soft tissue calcification.
Current therapy for secondary HPT includes oral phosphate binders, dietary P restriction, Ca supplementation and vitamin D therapy. Calcimimetic compounds such as AMG073 are compounds that mimic or potentiate the effects of extracellular calcium, and offer an alternative therapeutic approach for the treatment of HPT. AMG acts in an agonistic manner on the PT gland Ca receptor to increase the sensitivity of parathyroid cells to extracellular ionized calcium. Calcimimetics also act on parafollicular cells of the thyroid gland to increase calcitonin secretion. AMG has been shown to suppress PTH secretion and increase calcitonin release. A reduction in serum PTH is expected to lead to improvement of renal osteodystrophy and bone pathology.

## A. CLINICAL PLANS

The initial clinical trial (Amgen Protocol 980126) is a phase II double-blind, randomized, placebo-controlled, multicenter study to assess safety, tolerability, pharmacokinetics and clinical effects of single and multiple doses of AMG073. Measurements will be laboratory tests, ECGs, adverse events, physical examination, AMG073 pharmacokinetics, and plasma PTH, total serum Ca concentrations and serum calcitonin concentrations. Trial will be done in patients with secondary HPT.
Study will consist of a single dose phase, 72h follow up phase and washout phase of minimal 4 weeks, followed by a multiple dose phase of 8 days, plus 72h follow up phase. Five (5) single dose cohorts will consist of 10 subjects each (2 placebo, 8 on drug) for 5, 10, 25, 50, 75 mg doses. Cohorts will be sequential (ascending dose regimen), and SMC will evaluate whether it is safe to advance to next dose. Three (3) multiple dose cohorts will consist of 10 subjects each (2 placebo, 8 on drug) for 3 doses selected from 5, 10, 25, 50 and 75 mg. Fifty subjects will be in single-dose phase, thirty subjects will continue to multiple-dose phase. The latter will receive the same study medication as they received in the single-dose phase.
Previous human experience has been obtained in a double blind phase I study in normal volunteers. Single doses of 1, 5, 25, 50, 100 mg , and multiple doses of 25, 50, 100 mg daily for 8 consecutive days were administered did not cause serious adverse events. AMG caused a decrease in plasma PTH at single and multiple doses of 25, 50,

100 mg. A single 100 mg doses caused a decrease in serum Ca. In the multiple dosing study, 5 subjects receiving daily 100 mg doses were withdrawn due to mild hypocalcemia or related symptoms (section 9 of IND).
AMG is a second generation derivative of the parent compound R-568 [redacted]. R-568 has low bioavailability due to liver metabolism, and in humans there is large inter- and intrapatient variability in drug blood levels. Sponsor projects from chemistry and preclinical data that, compared to R-568, AMG-073 will have higher bioavailability and less blood level variability.

## B. CHEMISTRY

Chemical name for AMG 073.HCl is (N-[1-(R)-(1-naphthyl)ethyl]-3-[3-(trifluoromethyl)phenyl]-aminopropane.
AMG is registered as AMG 099073-01 in Amgen Chemical Registration File. AMG 073 is a calcimimetic intended to reduce plasma levels of PTH.

| | |
|---|---|
| Empirical formula: | $C_{22}H_{22}F_3N.HCl$ |
| Molecular weight: | 394 |
| Empirical formula free base: | $C_{22}H_{22}F_3N$ |
| Molecular weight free base: | 357 |
| Appearance: | White to off-white powder |
| Solubility: | Methanol or 95% ethanol, slightly soluble in water |
| Drug product: | [redacted] |
| Strength: | 5 or 25 mg |

## C. PHARMACOLOGY

**Efficacy Pharmacology**
In normal and hyperparathyroid rats, single oral doses of AMG (0.4-36 mg/kg) decreased serum PTH and Ca levels, and increased calcitonin. The effect was dose-dependent.

**Safety Pharmacology**
In guinea pigs, a single 20 mg/kg iv dose produced a transient increase in airway resistance and bronchoconstriction, leading to death in 1 animal. At a single oral dose of 200mg/kg, AMG increased gastric motility in mice by 35%.
AMG did not antagonize ACh, BaCl, or histidine-induced contractions of the guinea pig ileum at concentrations of 10 ug/ml. In mice, single oral doses of up to 200 mg/kg had no neurologic, analgesic, anti-convulsive, proconvulsive effects. In rats, single oral doses of up to 200 mg/kg had no effect on diuresis or body temperature.

## D. TOXICOLOGY

**Toxicity Studies**

Single or escalating dose studies

| Study Number | Species | Dosing schedule | Dosing Route | Doses (mg/kg) | N/sex /grp | Parameters | Findings |
|---|---|---|---|---|---|---|---|
| 970151 | rat | single dose | oral gavage | 0, 10, 100, 500 | 5 | BW, FC, signs, organ | Mortality: 500 mk: 1f dead on Day 4, cause unclear<br>BW/FC: 500 mk: BWG and FC slightly reduced |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | weights, gross path, histopath | during week 1 post-dosing |
| 970152 | rat | single dose | i.p. injecti on | 0, 1, 5, 20 | 5 | BW, FC, signs, organ weights, gross path, histopath | BW: 20 mk: BWG reduced in m during week 1 post dose.<br>Pathology: 5, 20 mk: adhesion of liver lobes, white discoloration of liver and/or spleen, capsulitis and capsule fibrosis of liver and spleen in several animals |
| 970153 | mouse | single dose | oral gavage | 0, 10, 100, 500 | 5 | BW, FC, signs, organ weights, gross path, histopath | Mortality and signs: 10, 100 mk: 1 f dead in each group on Day 8, with distended, air-filled intestines and stomach. 500 mk: 1m dead on Day 12, with decreased activity, abnormal signs and air-filled intestines and stomach. 1m with decreased activity on Day 8-10.<br>BW/FC: 500 mk: BWG and FC reduced in m on Day 8. |
| 970154 | mouse | single dose | i.p. injecti on | 0, 1, 5, 20 | 5 | BW, FC, signs, organ weights, gross path, histopath | Mortality and signs: 20 mk: 1m and 1f dead on Day 3 and 6, with decreased activity, abnormal signs, and distended dark red (m) or air-filled (f) intestines or stomach. 20 mk: signs in all animals on Day 1, in a few during week 1: decreased activity, abnormal gait and stance, body quiver/prostation.<br>Pathology; 0, 1, 5, 20 (all groups): liver areas of capsulitis and/or capsule fibrosis. |
| 970060 | dog | escalati ng dose schedul e (4-day multiple dosing plus ≥3-day washout periods) | oral gavage | 16, 32, 64, 96, 128, 160, 200 | 4 | signs, plasma ionized calcium and pH, PK | Signs: emesis (≥16mk), salivation (≥32mk), nonformed feces (≥16mk), tremors (≥64mk).<br>Serum Ca: All doses: Calcium levels reduced (22% at 200 mkd, at 2-8h postdose).<br>PK: 200 mkd: Cmax: ↓ AUC: 770 ng.h/ml,<br>$T_{1/2}$ 4.7h |
| 970142 | monke y | single escalati ng doses on days 1,3,5 | intrana sal gastric gavage | 16, 32, 48 | 1 | signs, BW, plasma calcium and pH | Signs: >32 mk: suppressed appetite<br>Serum Ca: All doses: ionized calcium suppressed by 25-30% (16 mk, 4h-12h postdose), 35-25% (32 mk, 6h postdose), 35-40% (48h, 12h postdose).<br>No effects on plasma pH. |

Multiple dose studies

| Study Number | Specie s | Dosing schedul e | Dosin g Route | Doses (mg/kg) | N/sex /grp | Findings |
|---|---|---|---|---|---|---|
| 970018 | rat | 14 days, daily | oral gavage | 0, 50, 250, 500 | 5 | Mortality: 250 mkd 1f, 500 mkd 5f, 3m.<br>Signs: 250, 500 mkd: stained fur, abnormal breathing, salivation, sneezing, eyes closed, pallor, weakness, tremors, dehydration, cold, thin, hunched posture, distention of abdomen, soft feces, reduced feces, reduced activity.<br>BW, FC: reduced at all doses<br>Ophtalmology: 250, 500mkd: cataracts in all surviving |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | Hematology/clin path: 50-500 mkd (m), 250 mkd (f): reduced Hb, RBC, Hct, WBC, elevated protrombin time, increase in BUN, increase in cholesterol.<br>Ca, PTH: All doses: dose-related reduction in serum calcium, with reduced predose levels. Reduction in PTH similar at all doses, with normal predose levels<br>Urine: 250, 500 mkd: WBC in urine in m and f<br>Organ weights: thymus reduced (250, 500), uterus reduced (250)<br>Gross findings: emaciation (>250), dilatation of GI (500)<br>Histopathology:<br>250, 500 mkd: myocardial degeneration, adrenal degeneration, kidney necrosis of cortical tubules, ovarian degeneration, uterine atrophy, lymphoid tissue necrosis/atrophy, salivary gland acinar hypertrophy , bone marrow hypocellularity<br>500 mkd: prostate atrophy, mucosal hyperplasia of colon/rectum<br>PK: non-linear absorption with relative decrease at higher doses<br>Values: 500 mkd (Day 14): Cmax = — AUC = 14700 ng.h/ml, $T_{1/2}$=12h<br>NOAEL <50 mkd |
| 970070 | rat | 1 month, daily | oral gavage | 0, 5, 50, 125 | | Mortality: 1 control, 1LD, 1HD.<br>Signs: 50, 125 mkd: stained fur, abnormal breathing, dehydration.<br>BW, FC: 50, 125 mkd: slightly reduced in f.<br>Ophtalmology: 50, 125mkd: cataracts.<br>Hematology/clin path: 50, 125 mkd: elevated protrombin time in f, elevated BUN, triglycerides at 125 mkd.<br>Ca, PTH: 50, 125 mkd: reduction in serum calcium >30 min postdose. Reduction in PTH at all doses.<br>Urine: 50, 125 mkd: increased Ca, decreased Na<br>Organ weights: uterus reduced (all doses)<br>Histopathology:<br>125mkd: myocardial inflammation<br>50, 125mkd: mucosal hyperplasia of cecum/colon<br>PK: Cmax and AUC increased less than proportional to dose. Ratio 125/5 mkd, Day 1: Cmax 10-fold, AUC 19-fold, Day 28: Cmax 11-fold, AUC 76-fold.<br>Values: 125 mkd (Day 28): Cmax = . — AUC = 6180 ng.h/ml, $T_{1/2}$ = 18h<br>NOAEL <5 mkd |
| 970078 | dog | 1 month, daily, 2 weeks recover y | oral gavage | 0, 5, 50, 100 | 6 or 4 | Signs: Emesis in MD, HD. Tremor and hypoactivity in HD.<br>Hematology: mild anemia in MD, HD.<br>Ca, PTH: decreased serum Ca in MD, HD, decreased ionized Ca in all treated, reduced PTH levels in all treated.<br>Urine: Urine volume increased in MD, HD, specific gravity decreased, pH decreased. Decreased Na, K, Cl. Increased Ca, or increased Ca excretion in all treated. After recovery urine volume remained increased and specific gravity decreased in HD.<br>Pathology: no remarkable findings<br>PK Values: 100 mkd (Day 28): Cmax= — AUC=503ng.h/ml, $T_{1/2}$=6h |
| 970147 | monke y | 7 days, daily | intrana sal gastric gavage | 0, 24, 48, 96 | 2 (mon keys not | Signs: Drug-related suppression of appetite at all doses. Emesis at 96 mkd in 2/2 animals.<br>No clear effects on hematology, clin path, urinalysis.<br>All doses: ionized calcium decreased pre-dose (ca. 15%) and |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | sacrificed) | post-dose, with lowest levels at 4-6h postdose (ca. 30% decrease). Levels were back to near-normal in 48h. No clear dose-relationship in calcium effect.<br>PK: Cmax and AUC increased less than proportional to dose (absorption less at high dose). Absorption erratic over time. BA estimated at 10%. $T_{1/2}$ 5-15h. Cmax (day 8) < Cmax (day 1) at MD, HD. No gender effects on PK.<br>Values: 96 mkd (Day 8): Cmax= —— , AUC 1330 ng.h/ml, $T_{1/2}$ 10h |
| | | | | | | |
| 960128 | Novascreen | 39 ug/ml | in vitro | effect on ligand-receptor binding | | AMG was active (>50% inhibition of binding) in following receptor systems:<br>alpha1-adrenergic, muscarinic, dopaminergic, sigma, sodium site 2, NK2 and dopamine and serotonine sites on transporter proteins |

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## E. TOXICOKINETICS

**General**

Assay of AMG-073 was done by HPLC. In rats, dogs and monkeys, oral clearance [(dose/AUC)x(1/F)] was very high indicating low oral bioavailability. This may be due to high first-pass metabolism. Mean T1/2 in all three species was less than 24h.

In rats, excretion and tissue distribution were similar following oral and i.v. dosing. Excretion of radioactively-labeled AMG was: 40% in feces, 20-25% in breathed CO2, 20-27% in urine. Dose was largely cleared after 96h. After 96h tissue:blood ratio's varied between 1 and 7.6. Largest tissue:blood radioactivity ratios were seen in adrenal gland (7.6)>liver (4.6)>adipose (4.5)>ovaries (3.3)> kidney (3), lowest one in muscle (1.01). In dogs, excretion of radiolabeled test substance was again similar after oral and i.v. dosing, and was 40%-70% in feces, and 12-32% in urine, consistent with high biliary excretion of radiolabelled drug and/or metabolites. Dose was largely cleared in 72h. T1/2(elim) of radioactivity was long: 58-162h. Plasma protein binding was large: 98-99% in mice, rats, dogs, monkeys and humans.

AMG 073 is predicted to be cleared mostly by oxidative hepatic metabolism by P450 enzymes. Microsomal clearance was high in mouse, rat, dog, monkey (>500 ul/min/mg) and in humans it was 93 ul/min/mg. One major metabolite -as yet unidentified- was seen in HPLC assay. AMG was a potent inhibitor of CYP2D6 (IC50<0.1 uM), and could thus slow the metabolism of other drugs.

**PK parameters**

1. Rat studies

PK 14-day multiple dose study

| Dose (mg) | Day | Cmax (ng/ml) | AUC (0-24h) ng.h/ml | T1/2 (h) | Accumulation Ratio(AUC Day14/AUC Day1) |
|---|---|---|---|---|---|
| 50 | 1 | | 982 | 4.7 | - |
| | 14 | | 941 | 10 | 0.96 |
| 250 | 1 | | 4930 | 23 | - |
| | 14 | | 6690 | 7.8 | 1.4 |
| 500 | 1 | | 4550 | 7 | - |
| | 14 | | 14700 | 12 | 3.2 |

PK 28-day multiple dose study

| Dose (mg) | Day | Cmax (ng/ml) | AUC (0-24h) ng.h/ml | T1/2 (h) | Accumulation Ratio(AUC Day14/AUC Day1) |
|---|---|---|---|---|---|
| 5 | 1 | | 126 | - | - |
| | 28 | | 81 | 2.4 | 0.64 |
| 50 | 1 | | 1110 | - | - |
| | 28 | | 1400 | 6.5 | 1.3 |
| 125 | 1 | | 2350 | - | - |
| | 28 | | 6180 | 18 | 2.6 |

2. Dog studies

PK 28-day multiple dose study

| Dose (mg) | Day | Cmax (ng/ml) | AUC (0-24h) ng.h/ml | T1/2 (h) | Accumulation Ratio(AUC Day28/AUC Day1) |
|---|---|---|---|---|---|
| 5 | 1 | | 9.0 | - | - |
| | 28 | | 21 | 2.4 | 0.79 |
| 50 | 1 | | 216 | - | - |
| | 28 | | 152 | 6.5 | 0.67 |
| 100 | 1 | | 440 | - | - |
| | 28 | | 503 | 18 | 1.6 |

3. Monkey studies
PK 28-day multiple dose study

| Dose (mg) | Day | Cmax (ng/ml) | AUC (0-24h) ng.h/ml | T1/2 (h) | Accumulation Ratio(AUC Day8/AUC Day1) |
|---|---|---|---|---|---|
| 24 | 1 | | 468 | 6 | - |
| | 8 | | 556 | 5 | 1.34 |
| 48 | 1 | | 1500 | 7 | - |
| | 8 | | 1170 | 9 | 0.86 |
| 96 | 1 | | 1930 | 15 | - |
| | 8 | | 1330 | 10 | 0.90 |

*Comment*:
In rats and monkeys nominal AUC values are roughly 10x $C_{max}$ values, while in dogs this factor is much lower (2-4x).

## F. GENOTOXICITY

Four mutagenicity assays were performed.

1. Ames assay in S. typhimurium TA98, TA100, TA1535, TA1537 and E.Coli WP2 uvrA in presence and absence of S-9
2. In vitro mammalian cell gene mutation assay (CHO cells, HGPRT) in absence and presence of S-9
3. In vitro mammalian chromosomal aberration test using CHO cells in absence and presence of S-9
4. In vivo mouse erythrocyte micronucleus test

AMG 073 was negative in all 4 assays.

## G. SUMMARY AND EVALUATION

Pharmacology
AMG-073 appears to suppress PTH secretion and serum calcium levels. This effect can be attributed to its calcimimetic action at the parathyroid gland calcium receptor. There are potential safety issues involving bronchoconstriction and GI motility.

Toxicology
In single dose studies, death occurred in rats at 500 mg/kg (oral). In mice, death occurred at 10, 100 and 500 mg/kg (oral), and at 20 mg/kg i.p. Clinical signs in mice that died included decreased activity and distended, air-filled or dark-red stomach and intestines. Decreased body weight gain or food consumption was seen in rats (500 mg/kg oral, 20 mg/kg ip), and monkeys (doses ≥ 32 mg/kg). Dogs experienced emesis and non-formed feces (≥16 mg/kg), salivation (≥32 mg/kg), and tremors (≥64 mg/kg).

In multiple dose studies in rats, death occurred at 250 mkd. Body weight was reduced at doses ≥50 mkd in both 14- and 28-day studies, and hematology parameters were abnormal at 50 mkd in 14-day study. Various other toxicities were observed at doses ≥ 250 mkd (14 days) or ≥50 mkd (28 days), including cataracts, increased BUN values, increased cholesterol, reduced uterus and thymus weights, emaciation and GI dilatation, myocardial abnormality, hyperplasia of GI mucosa, kidney cortex necrosis, bone marrow hypocellularity, lymphoid tissue necrosis. The decreases in serum Ca and PTH that occurred at all doses were the expected pharmacological effect of the compound.
In the 28-day multiple dose dog study, emesis, tremor and hypoactivity, and hematological and fluid homeostasis abnormalities were seen at doses ≥50 mkd.

In the 8-day multiple dose monkey study, suppressed appetite was seen at doses ≥24 mkd.

| Rat | Target organs | NOAEL | LOAEL | Adverse effects at LOAEL | Cmax, AUC at LOAEL |
|---|---|---|---|---|---|
| 14-day study (50, 250, 500 mkd) | CNS, eye, thymus, uterus, ovary, GI tract, heart, kidney, lymphoid tissue, bone marrow | <50 mkd | 50 mkd | BW loss, hematological effects | 941 |
| 28-day study (5, 50, 125 mkd) | CNS, eye, uterus, GI tract, heart | 5 mkd | 50 mkd | BW loss, hematology effects, cataracts, BUN increase, cholesterol increase, GI dilatation, uterus/thymusweight decrease, GI mucosa hyperplasia, myocardial abnormality, kidney cortex necrosis, bone marrow hypocellularity, lymphoid tissue necrosis | 1400 |

| Dog | Target organs | NOAEL | LOAEL | Adverse effects at LOAEL | Cmax, AUC at LOAEL |
|---|---|---|---|---|---|
| 28-day study (0, 5, 50, 100 mkd) | CNS, GI tract (bone marrow?) | 5 mkd | 50 mkd | Emesis, anemia, increased urine volume, decreased urine Na, K, Cl, increased urinary Ca excretion | I, 152 |

| Monkey | Target organs | NOAEL | LOAEL | Adverse effects at LOAEL | Cmax, AUC at LOAEL |
|---|---|---|---|---|---|
| 8-day study (24, 48, 96 mkd) | CNS, GI tract | <24 mkd | 24 mkd | suppressed appetite | I, 556 |

Carcinogenicity and reproductive toxicity studies are planned but have not been carried out.

Genotoxicity

AMG 073 had no genotoxic activity in the Ames test, the mammalian cell gene mutation test, a chromosomal aberration test, and the mouse micronucleus test.

Clinical PK data

1. Single dose study

Doses: 1, 5, 25, 50, 100 mg

Doses of 5mg and above caused a transient, dose-dependent suppression of plasma PTH, with dose-dependent suppression duration. Baseline PTH averaged 20-25 pg/ml. Suppressed values at nadir (ca. 2h post-dose) ranged from [redacted] dose. Nadir level was [redacted] (BLQ) at doses >50 mg. A transient rebound above baseline occurred. Doses of 50 and 100 mg caused a reduction in serum Ca (maximum decrease -5% and -8% at 12-18h post-dose).

PK Single dose study

| Dose (mg) | Cmax (ng/ml) | Tmax (h) | AMG plasma concentration (0-24h average) (ng/ml) | $AUC_{(0-24h)}$ (ng.h/ml) |
|---|---|---|---|---|
| 5 | [illegible] | 1.3 | 0.077 | 1.85 |
| 25 | [illegible] | 2 | 0.82 | 19.7 |
| 50 | [illegible] | 2 | 2.6 | 62 |
| 100 | [illegible] | 1.5 | 8 | 192 |

2. Multiple dose study
Doses: 25, 50, 100 mg
Duration: 8 consecutive days

Plasma PTH was suppressed dose-dependently for up to 12h following dose. At 100 mg nadir value was below BLQ. There was a rebound in PTH above baseline at ca 12h post-dose.. 25 and 50 mg daily caused small reductions in serum Ca of 1-2%. At 100 mg daily dosing, a marked 15-20% reduction in serum calcium was obtained after ca 72h (mild hypocalcemia). Serum Ca returned to normal after 48h of dose discontinuation.

PK multiple dose study

| Dose (mg) | Day | Cmax (ng/ml) | $AUC_{(0-24h)}$ ng.h/ml | $T_{1/2}$ (h) | Accumulation Ratio(AUC Day7/Day1) |
|---|---|---|---|---|---|
| 25 | 1 | [illegible] | 19.4 | - | - |
| | 7 | | 36.4 | 37 | 2 |
| 50 | 1 | [illegible] | 71.2 | - | - |
| | 7 | | 128 | 35 | 1.7 |
| 100 | 1 | [illegible] | 163 | - | - |
| | 7 | | 184 | 43 | 0.98 |

Evaluation of clinical plans
The clinical protocol proposed in the IND is a phase II study in patients with secondary HPT. The largest single and multiple dose to be used is 75 mg. To estimate the human plasma levels PK data from previous phase I clinical studies with AMG are available. From these data (see above), it can be extrapolated that at single/multiple doses of 75 mg the plasma levels (AUC) in humans are expected to average approximately 150 ng.h/ml. This suggests a safety margin of 6x, 1x, 4x, based on toxicity data from rats, dogs and monkeys, respectively. Based on Cmax values the safety margin for the 75 mg dose is 6x, 2x, 4x.

| human data at 75 mg dose | | animal data (rat, dog, monkey) at LOAEL | | safety margin based on Cmax | safety margin based on AUC |
|---|---|---|---|---|---|
| Cmax | AUC | Cmax | AUC | | |
| [illegible] | 150 | [illegible] | 941, 152, 556 | 6x, 2x, 4x | 6x, 1x, 4x |

In conclusion, the highest dose to be used (75 mg) appears to be reasonably safe. Main toxicities to be expected on the basis of the animal studies are nausea, vomiting, anemia. Other toxicities suggested by animal studies are GI tract, kidney, heart, eye, hematopoietic tissue, reproductive organ toxicities. In the initial and future clinical studies these toxicities should be kept in mind, and appropriate monitoring should be conducted.

## H. RECOMMENDATION

Pharmacology has no objection to initiation of the clinical protocol described in this IND.

/S/

Gemma A. Kuijpers

**Executive CAC**
Date of Meeting: December 16, 2003

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Robert Osterberg, Ph.D., HFD-520, Alternate Member
Karen Davis-Bruno, Ph.D., Team Leader
Gemma Kuijpers, Ph.D., Presenting Reviewer

Author of Draft: Gemma Kuijpers

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

**NDA #: 21-688**
Drug Name: Cinacalcet HCl (Sensipar™)
Sponsor: Amgen Inc.

Background: Cinacalcet HCl (AMG-073) is a calcimimetic that binds to the calcium receptor on the parathyroid gland. Its pharmacologic effect is a suppression of PTH secretion and reduction of serum calcium levels. The proposed indication is treatment of secondary hyperparathyroidism in patients with end stage renal disease and treatment of primary hyperparathyroidism. The compound is not genotoxic.

Mouse Carcinogenicity Study
A 104-week carcinogenicity study was carried out in CD-1 mice at doses of 0, 0, 15, 50, 125 mg/kg/day (males), and 0, 0, 15, 70, 200 mg/kg/day (females) (N=60/sex/grp, two control groups), by the dietary route. Doses were selected based on data from a 3-month dietary study in which a dose-related decrease in body weight gain was observed. In the 104-week study, a dose-related decrease in body weight was observed in both sexes of up to 35% (males) and 40% (females). There was no significant effect on survival. Exposure was dose-related with metabolite levels (M7) 10 to 20 times higher than those of parent drug. Effects related to the pharmacodynamic action of the drug (hypocalcemia, hyperphospatemia, soft tissue mineralization) were seen in the treated groups.
In high dose males, there was an increased incidence of kidney tubular adenoma ($Ctrl_{1+2}$-LD-MD-HD: 0-0-0-2). The finding was not statistically significant according to Sponsor's analysis (pairwise comparison: Ctrl-HD), or CDER Biometrics' analysis (trend test). Historical control incidences for tubular cell adenoma in males ranged from 0-5.1%.
In high dose females there were increased incidences of erythroid leukemia ($Ctrl_{1+2}$-LD-MD-HD: 0-0-0-2) and pituitary adenoma (intermediate lobe) ($Ctrl_{1+2}$-LD-MD-HD: 0-0-0-2), not statistically significant according to Sponsor's pairwise comparison or CDER Biometrics' trend test. The incidence of pituitary (intermediate lobe) hyperplasia was also increased in a dose-related and statistically significant manner. Uterine stromal sarcoma incidence was non-statistically significantly increased in mid dose females ($Ctrl_{1+2}$-LD-MD-HD: 3-1-4-1). Historical control incidences for pituitary adenoma intermediate lobe in females ranged from

0-0.9% (average 0.1%), and for uterine stromal sarcoma from 0-2.5% (average 1.8%). Erythroid leukemia was not observed in the historical control database (0/959). However, it was also observed in 1/60 low dose males in the current study.

Rat Carcinogenicity Study

A 104-week study was carried out in Sprague Dawley rats at doses of 0, 0, 5, 15, 35 mg/kg/day (males), and 0, 0, 5, 20, 50/35 mg/kg/day (females) (N=60/sex/grp, 2 control groups), by the dietary route. Doses were selected based on data from a 3-month dietary study in which a dose-related decrease in body weight gain was observed. In the 104-week carcinogenicity study, a dose-related decrease in body weight was observed in both sexes of up to 25% (males) and 35% (females). The female high dose had been lowered from 50 mg/kg/day to 35 mg/kg/day in Week 63 due to excessive body weight effects. There was no significant effect on survival. Exposure was dose-related with metabolite levels (M7) at least 20 times higher than those of parent drug. Effects related to the pharmacodynamic action of the drug (hypocalcemia, hyperphospatemia, soft tissue mineralization) were seen in the treated groups.

In high dose males, there was an increased incidence of combined malignant lymphoma ($Ctrl_{1+2}$-LD-MD-HD: 1-1-1-3) and an increased incidence of lung bronchio-alveolar carcinoma ($Ctrl_{1+2}$-LD-MD-HD: 0-0-0-2), The findings were not statistically significant according to Sponsor's analysis (pairwise comparison: Ctrl-HD), or CDER Biometrics' analysis (trend test). There was also an increase in bronchio-alveolar hyperplasia in the mid dose males. Historical control incidences for lung bronchio-alveolar carcinoma in males ranged from 0-1.7%, and for combined lymphoma from 0-8%. There was a single incidence of ependymoma in HD males, a tumor not observed in the historical control database (n=1224).

In high dose females there was an increased incidence of combined malignant lymphoma ($Ctrl_{1+2}$-LD-MD-HD: 0-1-0-3), which was not statistically significant according to Sponsor's pairwise comparison, but was statistically significant according to CDER Biometrics' trend analysis based on the concurrent control incidence of 0/60 (p=0.0163). Historical control incidence for lymphoma in females ranged from 0-4% (average 1.4%).

Executive CAC Recommendations and Conclusions:

Mouse Study:

- The Committee concluded that the high dose was adequate based on body weight effects and the study was acceptable.
- The Committee was concerned about the high incidence of convulsions in the control animals.
- The Committee concluded that the increased incidence of kidney tubular cell adenoma in high dose males was probably not biologically significant.
- The Committee concluded that the increased incidence of uterine stromal sarcoma in mid dose females was probably not biologically significant.
- The Committee noted that the incidences of erythroid leukemia and pituitary intermediate lobe adenoma and hyperplasia in the high dose females were clearly above historical control range indicating possible biological significance and potential treatment association. However, the Committee did not recommend that this finding be included in

the label because of the low incidence and the lack of statistical significance. The Committee recommended that the findings should be noted in the NDA review.

Rat Study:

- The Committee concluded that the high dose was adequate based on body weight effects and the study was acceptable.
- The Committee concluded that the increased incidence of lymphoma in high dose males was within historical control range and probably not biologically significant.
- The Committee concluded that the single incidence of ependymoma in high dose males, although above historical control range, was probably not treatment-related.
- The Committee noted that the incidence of lung bronchio-alveolar carcinoma adenoma in high dose males was above historical control range indicating possible biological significance and potential treatment association. However, the Committee did not recommend that this finding be included in the label because of the low incidence and the lack of statistical significance. The Committee recommended that the lung carcinoma finding in male rats should be noted in the NDA review.
- The Committee noted that the incidence of combined malignant lymphoma in female rats was in the upper range of historical control incidences. The Committee also noted that the historical control incidence of 1.4% indicated that this is a common tumor and the finding is not statistically significant according to trend test for common tumors (control incidence >1%). In addition, the increased incidence was observed at the high dose, which had been lowered during the study since it exceeded the MTD. The Committee recommended that the lymphoma finding in female rats should be noted in the NDA review, but need not be included in the label.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, HFD-510
/KDavisbruno, Team leader, HFD-510
/GKuijpers, Reviewer, HFD-510
/RHedin, CSO/PM, HFD-510
/ASeifried, HFD-024

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

A-8

Memo
To: NDA 21-688 Sensipar (Cinacalcet) Amgen
From: Karen Davis-Bruno; Ph.D. Supervisory Pharmacologist
Date: 2/10/04
Re: Proposed Pharm/Tox NDA labeling

Carcinogenesis, Mutagenesis, and Impairment of Fertility

Carcinogenesis: Standard lifetime dietary carcinogenicity bioassays were conducted in mice and rats. Mice were given dietary doses of 15, 50, 125 mg/kg/day in males and ——— 'day in females (exposure 2 times a human oral dose of 180 mg/day based on AUC comparison). Rats were given dietary doses of ——— /day in males and 5, 20, 35 mg/kg/day in females (exposures 2 times a human oral dose of 180 mg/day based on AUC comparison). No increased incidence of tumors was observed following treatment with cinacalcet.

Mutagenicity: Cinacalcet was not genotoxic in the Ames bacterial mutagenicity assay or in the Chinese Hamster Ovary (CHO) cell HGPRT forward mutation assay and CHO cell chromosome aberration assay, with and without metabolic activation or in the *in vivo* mouse micronucleus assay.

Impairment of Fertility: Female rats were given oral gavage doses of 5, 25, 75 mg/kg/day beginning 2 weeks before mating and continuing through gestation day 7. Male rats were given oral doses 4 weeks prior to mating, during mating (3 weeks) and 2 weeks post-mating. [redacted]

Pregnancy Category C
In pregnant female rats given oral gavage doses of 2, 25, 50 mg/kg/day during gestation no teratogenicity was observed at doses up to 50 mg/kg/day (exposure 4 times a human oral dose of 180 mg/day based on AUC comparison). Decreased fetal body weight was observed at all doses ( ——— less than a human oral dose of 180 mg/day based on AUC comparisons) in conjunction with maternal toxicity (decreased food consumption and body weight gain).

In pregnant female rabbits given oral gavage doses of 2, 12, 25 mg/kg/day during gestation , ——— no adverse fetal effects observed (exposures less than a human ——— dose of 180 mg/day based on AUC comparisons). Reductions in maternal food consumption and body weight were seen at doses of 12 and 25 mg/kg/day.

In pregnant rats given oral gavage doses of 5, 15, 25 mg/kg/day during gestation through lactation no adverse fetal or pup (post-weaning) effects were observed at 5 mg/kg/day (exposures less than a human therapeutic dose of 180 mg/day based on AUC comparisons) ——— . Higher doses of 15 and 25 mg/kg/day (exposures 2 times a human ——— dose of 180 mg/kg/day based on AUC comparisons) were accompanied by maternal [redacted]

There are no adequate and well controlled studies in pregnant women. Sensipar should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Lactating Women:
Studies in rats have shown that Sensipar is excreted in the milk with a high milk to plasma ratio. It is not known whether this drug is excreted in human milk. [redacted] or to discontinue the drug, taking into account the importance of the drug to the lactating woman.

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

/s/

Gemma Kuijpers
2/13/04 04:11:17 PM
PHARMACOLOGIST

maybe this worked??

Karen Davis-Bruno
2/13/04 04:13:53 PM
PHARMACOLOGIST
supervisory concurrence, see TL memo/label

Statistical Review and Evaluation of Rat and Mouse Carcinogenicity Studies

| | |
|---|---|
| NDA: | 21-688 |
| Applicant: | Amgen Inc.<br>Thousand Oaks, California |
| Name of Drug: | Sensipar (cinacalcet HCl) |
| Documents Reviewed: | Electronic Submission, Dated August 14, 2003<br>Data submitted electronically |
| Reviewing Pharmacologist: | Gemma Kuijpers, Ph.D. |
| Statistical Primary Reviewer: | Joan Buenconsejo, MS, MPH |
| Statistical Secondary Reviewer: | Karl Lin, Ph.D. |
| Biometrics Division Director: | S. Edward Nevius, Ph.D. |

## Background

In this NDA submission, two-year carcinogenicity studies in two rodent species, one in CD-1 mice and one in Sprague-Dawley rats, were conducted. These studies were intended to assess the carcinogenic potential of the test article, AMG-099073-01, a calcium mimetic intended for use as a treatment of hyperparathyroidism, following oral administration by the dietary route for 104 weeks.

## Study Design

The designs of the carcinogenicity studies were similar with primary differences arising in the dose levels and rodent species. The current review evaluates and presents results separately for each species.

### *1. Study in Mice (Amgen Reference No. 100250)*

Two separate experiments, one in male and one in female were conducted. In each of these two experiments, there were two control groups and three treatment groups. Three hundred males and three hundred females were assigned to control and treated groups of equal size. Groups of 60 males and 60 females were given AMG-099073-01 in the diet for 104 weeks at concentrations formulated to achieve dose levels of 0, 0, 15, 50 and 125 mg/kg/day in males and 0, 0, 30, 70, and 200 mg/kg/day in females (Table 1). Note that the control groups were given rodent diet alone.

Body weight and food consumption were recorded weekly from one week prior to the start of treatment and for the first 13 weeks of treatment and then every 4 weeks thereafter until the end of the study. Water consumption was monitored by visual inspection throughout the study. Clinical signs were monitored daily and a detailed clinical examination and palpation was carried out once each week. An ophthalmoscopic examination was conducted once prior to the start of treatment and again during weeks 26, 53, 78 and 102. Routine haematology and clinical chemistry parameters were assessed during week 105/106.

All animals were sacrificed by exposure to carbon dioxide, followed by exsanguination. Following completion of 104 weeks of treatment, all surviving animals were killed and subjected to necropsy where a full list of organs and tissues were weighed/collected and examined. The animals were not fasted overnight prior to necropsy. A terminal body weight was taken from each animal undergoing necropsy. In a few cases the terminal body weight was not recorded, in error. This protocol deviation was not considered to have affected the integrity of the study. All animals, including dead or moribund animals underwent a gross necropsy. Dead or moribund animals were necropsied as soon as possible after discovery.

*2. Study in Rats (Amgen Reference No. 100209)*

Two separate experiments, one in male and one in female were conducted. In each of these two experiments, there were two control groups and three treatment groups. Three hundred males and three hundred females were assigned to control and treated groups of equal size. Groups of 60 males and 60 females were given AMG-099073-01 in the diet for 104 weeks at concentrations formulated to achieve dose levels of 0, 0, 5, 15, and 35 mg/kg/day in males and 0, 0, 5, 20, and 50 mg/kg/day in females (Table 1).

After review of the body weight data and following discussions between the Sponsor and the Executive Carcinogenecity Assessment Committee (CAC) of the Agency, the dose level for High dose females was reduced from 50 mg/kg/day to 35 mg/kg/day on week 63 of treatment. Note that the control groups were given rodent diet alone.

Body weight and food consumption were recorded weekly from one week prior to the start of treatment and for the first 13 weeks of treatment and then every 4 weeks thereafter until the end of the study. Water consumption was monitored by visual inspection throughout the study. Clinical signs were monitored daily and a detailed clinical examination and palpation was carried out once each week. An ophthalmoscopic examination was conducted once prior to the start of treatment and again during weeks 26, 53, 78 and 102. Routine haematology and clinical chemistry parameters were assessed during week 105/106.

All animals were sacrificed by exposure to carbon dioxide, followed by exsanguination. Following completion of 104 weeks of treatment, all surviving animals were killed and subjected to necropsy where a full list of organs and tissues were weighed/collected and examined. The animals were not fasted overnight prior to necropsy. A terminal body weight was taken from each animal undergoing necropsy. In a few cases the terminal body weight was not recorded, in error. This protocol deviation was not considered to have affected the integrity of the study. All animals, including dead or moribund animals underwent a gross necropsy. Dead or moribund animals were necropsied as soon as possible after discovery.

## Sponsor's Analysis and Results

Mortality data has been presented graphically using Kaplan-Meier survival curves and pairwise comparisons using Wilcoxon rank sum test modified censored survival data. *P*-values for the

multiple comparisons are reported. Their result showed no statistically significant differences in male and female mortality between the combined Control groups and dose groups.

Histological incidence data were analyzed using a Fisher's test (two-tailed) with pairwise comparisons between the combined control groups (Control I and II) and each of the other groups.

Sponsor concluded that the nature, incidence and distribution of tumors recorded in all groups of all sexes and in both species did not give any indication of treatment induced carcinogenesis.

## Statistical Analysis Methods

This reviewer conducted an independent analysis on the carcinogenicity data submitted by the Sponsor. The analysis conformed to the Food and Drug Administration's Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (May, 2001). In addition, this reviewer's analysis was primarily conducted using *e*Review of Animal Carcinogenicity, a review tool developed for and utilized by CDER reviewers.

Mortality Analysis

Tests for homogeneity and dose mortality trends were conducted using survival analysis methods described by Cox (1972) and the Kruskal-Wallis Test (Gehan, 1965; Breslow, 1970; Thomas, Breslow, and Gart, 1977) where the latter test weights early failures more heavily.

Tumor Data Analysis (Trend Test)

This reviewer conducted the trend tests on tumor incidence rates using the method described by Peto et. al. (1980) and the method of exact permutation trend test developed by the Division of Biometrics II. The sponsor classified tumors as fatal, possibly fatal, incidental, or possibly incidental, in which case, this reviewer combined fatal and possibly fatal as one group called fatal, and combined incidental and possibly incidental in another group called incidental. Data of incidental and fatal tumors were analyzed via the prevalence and death-rates methods, respectively. A combined test was used to analyze tumors classified as both fatal and incidental. The method of exact permutation trend test was used to counter underestimation of p-values when tumor incidence across the treatment group was small. All tests are performed separately for males and females for both species.

Multiple Testing Adjustment

A rule proposed by Haseman (1983) could be used to adjust the effect of multiple testings. A similar rule proposed by the Divisions of Biometrics, CDER/FDA was used in this review. The rule states that in order to keep the overall false-positive rate at the nomial level of approximately ten percent, tumor types with a spontaneous tumor rate of no more than one percent should be tested at 0.025 level, otherwise the level should be set at 0.005. (Lin, 1995, 1997; Lin and Rahman, 1998a, 1998b) The 10 percent overall false positive rate is seen by CDER statisticians as appropriate in a new drug regulatory setting.

Evaluation of Validity of the Design of the Study

An evaluation of validity of the study design was conducted in a negative study (that is, an analysis did not indicate any tumor type with a significant positive linear trend) before drawing the conclusion that the drug was not carcinogenic in rodents. It is important to look into the following two issues in the evaluation as pointed out in the paper by Haseman (1984). Two issues are:

1. Were enough animals exposed, for a sustained amount of time, to the risk of late developing tumor?
2. Were dose levels high enough to pose a reasonable tumor challenge to the animals?

There is no consensus among experts regarding the number of animals and length of time at risk, although most carcinogenicity studies are designed to run for two years with fifty animals per treatment group.

The following are some rules of thumb regarding these two issues as suggested by the experts in this field:

For the first issue, Haseman (1999) suggested that, as a rule of thumb, a 50% survival of 50 initial animals in the high dose group, between weeks 80-90, would be considered as a sufficient number of animals under an adequate exposure. In addition, Chu, Cueto, and Ward (1981), suggested that "To be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have groups of animals with greater than 50% survival at one-year."

Regarding the issue of adequate dose levels, it is generally accepted that the high dose should be close to the MTD (maximum tolerated dose). Based from the paper of Chu , Cueto and Ward (1981), only one of the following criteria is needed to justify that the high dose is close to MTD, and they are:

1. If there is a detectable loss of weight gain of up to 10% in a dosed group relative to the controls; or
2. If dosed animals exhibit clinical signs or severe hisyopathologic toxic effects attributed to the chemical; or
3. If the dosed animals show a slightly increased mortality compared to the controls.

## Results and Discussion

### *1. Study in Mice (Amgen Reference No. 100250)*

Survival Analysis: The intercurrent mortality data are given in Tables 2A and 2B for males and females, respectively. The Kaplan-Meier curves for death rate are given in Figures 1A and 1B for males and females, respectively. The homogeneity of survival was tested separately for males and females using the Cox test (Cox, 1972) and the Generalized Wilcoxon test (Gehan, 1965). Results of the tests are presented in Tables 3A and 3B for males and females, respectively. The tests showed no significant difference in survivals across treatment groups in both sexes. No statistically significant increment in mortality in any of the treated groups compared to the concurrent control group in both sexes is found.

Tumor data analysis: As explained in the methods section, tumor types has been reclassified by this reviewer by assigning fatal tumor to 'tumor caused death' and 'tumor probably caused death', and incidental tumor to 'tumor did not cause death' and 'tumor probably did not cause death'. Following Peto et al., (1980) this reviewer applied the 'death rate method' and the 'prevalence method' on these two new categories of tumors, respectively. For tumor types occurring in both categories, a combined test of 'death rate method' and the 'prevalence method' was performed. For the calculation of $p$ values, the exact permutation method and the approximation method based on normal distribution were used. Since the mortality rates were not different for the two control groups, they were pooled together. Scores used were 0, 15, 50, and 125 (equivalent to the dose levels) for the pooled control, low, medium, and high dose groups, respectively in male mice, and 0, 30, 70, and 200 for the pooled control, low, medium, and high dose groups, respectively in female mice. The time intervals used were 0 – 52, 53 – 78, 79 – 91, 92 – 104 weeks and terminal sacrifice. The tumor incidence rates and the tumor types with asymptotic $p$-values less than 0.05 for dose-response relationships are listed in Tables 4A, 4B and 4C for males, females, and females with some combined tumors, respectively. As can be seen from the tables, on the basis of the Division's $p$-value adjustment rule, no significant positive trend was observed. Combining tumor types within an organ such as adenoma anterior lobe and adenoma intermediate lobe in the pituitary gland, as well as stromal polyp and stromal sarcoma in the uterus, also provide no significant positive trend.

Evaluation of the validity of the design of the mouse study: In light of the criteria presented in the Statistical Analysis section method of this review, we will now investigate the validity of the experimental design of the mouse carcinogencity study. Table 5 presents the summary of survival data on mice in the high dose group at different time intervals. Based on the survival criterion Haseman proposed, it could be concluded that enough mice in both sexes were exposed to the drug for a sufficient amount of time as more than 50% of the animals in male and females were alive in the high dose group between weeks 80 and 90.

In Table 6, we present the summary of body weight gains data in the mice study. Meanwhile, the result in Table 7 shows that relative to the controls, the high dose group had 41.7% decrement of body weight gain in females, while males in high dose group had 35.9%

decrement of body weight gain. The decrease in body weight gain in the high dose group in both sexes could be due to excessive dose level.

The mortality rate at the end of the experiment is shown in Table 8. The mortality rate at the high dose group was lower than that of the controls for male mice. Meanwhile, the high dose group is the same compared to control group 1, but lower compared to control group 2 for female mice. The decreased mortality rate in male mice on the high dose group suggests an inadequacy of the high dose level. On the other hand, there is also a possibility that the high dose level is inadequate in the female mice because one of the control groups has slightly higher mortality rate than the high dose group.

In conclusion, as observed from the mortality data, the high dose level used by both sexes might not reach the MTD level. However, the body weight gain data suggested that the dose level used in the high dose group might be over MTD. Therefore other clinical signs and histopathological toxic effects should also be considered in the evaluation of the adequacy of the doses used.

### 2. *Study in Rats (Amgen Reference No. 100209)*

Survival Analysis: The intercurrent mortality data are given in Tables 9A and 9B for males and females, respectively. The Kaplan-Meier curves for death rate are given in Figures 2A and 2B for males and females, respectively. The homogeneity of survival was tested separately for males and females using the Cox test (Cox, 1972) and the Generalized Wilcoxon test (Gehan, 1965). Results of the tests are given in Tables 10A and 10B for males and females, respectively. The tests showed departure from trend across treatment groups ($P = 0.02$) in male rats. The results also showed significant differences in survivals across treatment groups in male rats ($P = 0.04$).

Tumor data analysis: As explained in the methods section, tumor types has been reclassified by this reviewer by assigning fatal tumor to 'tumor caused death' and 'tumor probably caused death', and incidental tumor to 'tumor did not cause death' and 'tumor probably did not cause death'. Following Peto et al., (1980) this reviewer applied the 'death rate method' and the 'prevalence method' on these two new categories of tumors, respectively. For tumor types occurring in both categories, a combined test of 'death rate method' and the 'prevalence method' was performed. For the calculation of $p$ values, the exact permutation method and the approximation method based on normal distribution were used. Scores used were 0, 5, 15, and 35 (equivalent to the dose levels) for pooled control, low, medium, and high dose groups, respectively in male rats, and 0, 5, 20, and 50/35 for pooled control, low, medium, and high dose groups, respectively in female rats. In the analysis of female rats, high dose groups are tested using dose levels 42.5 (average), 50 (starting dose), and 35 (ending dose). The time intervals used were 0 – 52, 53 – 78, 79 – 91, 92 – 104 weeks and terminal sacrifice. Because the results in the high dose groups for female rats using three different dose levels are almost identical, only the result from the average dose (42.5) in the high dose group is presented. The tumor incidence rates and the tumor types with asymptotic $p$-values less than 0.05 for dose-response relationships are listed in Tables 11A, 11B, 11C, and 11D(i) for males, males with combined tumors, females, and females with combined

tumors, respectively. As can be seen from the tables, on the basis of the Division's $p$-value adjustment rule, no significant positive trend was observed in both sexes except when combination of tumors is performed in the female rats. This observation is the same across different dose levels used in the high dose group. When lymphoma tumor and lymphoma lymphocytic were combined within the haemopoietic organ system in the female rats, there is a significant positive trend detected ($p = 0.0163$) in lymphoma tumor in haemopoietic organ.

As per the request of the pharmacologist, this reviewer performed additional trend analyses on female rats using two sets of data:
Set 1. Control 1, low, medium, high dose groups
Set 2. Control 2, low, medium, high dose groups

The results of the trend analyses are presented in Tables 11D(ii) and 11D(iii) for the two sets of data. As can be seen from the tables, on the basis of the Division's $p$-value adjustment rule, no significant positive trend was observed using either the set with control 1 group or the set with control 2 group.

Evaluation of the validity of the design of the mouse study: In light of the criteria presented in the Statistical Analysis section method of this review, we will now investigate the validity of the experimental design of the rat carcinogencity study. Table 12 presents the summary of survival data on rats in the high dose group evaluated at different time intervals. Based on the survival criterion Haseman proposed, it could be concluded that enough mice in both sexes were exposed to the drug for a sufficient amount of time as more than 50% of the animals in male and females were alive in the high dose group at the time points evaluated.

In Table 13, we present the summary of body weigh gains data in the rats study. Meanwhile, the result in Table 14 shows that relative to the controls, the high dose group had 35.1% decrement of body weight gain in females, while males in high dose group had 27.6% decrement of body weight gain. Similar to the mouse study, the results showed that the decrease in body weight gain in the high dose groups compared to the control groups could be due to excessive dose levels used.

The mortality rate at the end of the experiment is shown in Table 15. The mortality rate at the highest dose group is lower compared to control 1 but higher compared to control 2 in male rats. Meanwhile, highest dose group in females is lower relative to the controls. These decreased in mortality rates in the high dose group relative to the controls for female rats might suggest an inadequacy of the high dose. On the other hand, because there is inconsistency on the results in the male rats, making a definite conclusion is not possible.

In conclusion, as observed from the mortality data, the high dose levels used by female rats might not reach the MTD level. Meanwhile, from the mortality data on male rats, it is difficult to determine whether the high dose levels are adequate or not. On the other hand, the body weight gain data suggest that the dose level used in the high dose group might be over MTD. Therefore other clinical signs and histopathological toxic effects should also be considered in the evaluation of the adequacy of the doses used.

## Summary

In this submission report, animal carcinogenicity studies in rats and in mice were included. These studies were intended to assess the carcinogenic potential of AMG-099073-01 in rats and mice with appropriate drug levels for about 104 weeks.

Mice Study: This study had two control groups and 3 treatment groups (dose levels: 15, 50, 125 mg/kg/day in males and 30, 70, 200 mg/kg/day in females). Test results showed no statistically significant differences in survival across treatment groups in both sexes. Tests on tumor data showed no statistically significant dose-response in any of the tested tumor types in both sexes.

Rat Study: This study had two control groups and 3 treatment groups (dose levels: 5, 15, 35 mg/kg/day in males and 5, 20, 50/35 mg/kg/day in females). Test results showed statistically significant differences in survival across treatment groups, as well as statistically significant departure from trend in the male rats. However, the tests did not show statistically significant dose-response in tumor incidence in male rats. Meanwhile, the results showed no statistically significant differences in survival across treatment groups in female rats. However, when lymphoma tumor and lymphoma lymphocytic were combined within the haemopoietic organ system in the female rats, there is significant positive trend detected ($p = 0.0163$) in lymphoma tumor in haemopoietic organ.

From the mortality data, it can be concluded that the dose level used by the high dose group did not reach MTD possibly in both species and both sexes. However, the body weight gain data from both sexes and species suggested that the dose level in the high dose group was over MTD. Therefore other clinical signs and histopathological toxic effects should also be considered in the evaluation of the adequacy of the doses used.

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## Tables

Table 1: Dose levels (mg/kg/day) and animal distribution

| | Mice | | Rats | |
|---|---|---|---|---|
| Group | Male N=300 | Female N=300 | Male N=300 | Female N=300 |
| Control 1 | 0 | 0 | 0 | 0 |
| Control 2 | 0 | 0 | 0 | 0 |
| Low | 15 | 30 | 5 | 5 |
| Medium | 50 | 70 | 15 | 20 |
| High | 125 | 200 | 35 | 50/35 |

Table 2A: Intercurrent Mortality Rate Male Mice

| Week | Control 1 | | Control 2 | | 15 mg/kg/day | | 50 mg/kg/day | | 125mg/kg/day | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % |
| 0 – 52 | 3 | 5.0 | 2 | 3.3 | 3 | 5.0 | 8 | 13.3 | 3 | 5.0 |
| 53 – 78 | 9 | 20.0 | 5 | 11.7 | 8 | 18.3 | 6 | 23.3 | 7 | 16.7 |
| 79 – 91 | 7 | 31.7 | 7 | 23.3 | 5 | 26.7 | 5 | 31.7 | 8 | 30.0 |
| 92 – 103 | 9 | 46.7 | 11 | 41.7 | 9 | 41.7 | 6 | 41.7 | 6 | 40.0 |
| Terminal Sacrifice | 32 | 53.3 | 35 | 58.3 | 35 | 58.3 | 35 | 58.3 | 36 | 60.0 |

Table 2B: Intercurrent Mortality Rate Female Mice

| Week | Control 1 | | Control 2 | | 15 mg/kg/day | | 50 mg/kg/day | | 125mg/kg/day | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % |
| 0 – 52 | 8 | 13.3 | 5 | 8.3 | 7 | 11.7 | 5 | 8.3 | 5 | 8.3 |
| 53 – 78 | 10 | 30.0 | 15 | 33.3 | 16 | 38.3 | 15 | 33.3 | 10 | 25.0 |
| 79 – 91 | 5 | 38.3 | 5 | 41.7 | 6 | 48.3 | 8 | 46.7 | 11 | 43.3 |
| 92 – 103 | 14 | 61.7 | 14 | 65.0 | 7 | 60.0 | 11 | 65.0 | 11 | 61.7 |
| Terminal Sacrifice | 23 | 38.3 | 21 | 35.0 | 24 | 40.0 | 21 | 35.0 | 23 | 38.3 |

Table 3A: Intercurrent Mortality Comparison Male Mice

| | Method | | | |
|---|---|---|---|---|
| | Cox | | Kruskal-Wallis | |
| | Statistics | P-Value | Statistics | P-Value |
| Time-Adjusted Trend Test | | | | |
| Depart from Trend | 0.5800 | 0.9010 | 0.9455 | 0.8144 |
| Dose-Mortality Trend | 0.1007 | 0.7510 | 0.0171 | 0.8961 |
| Homogeneity | 0.6807 | 0.9537 | 0.9625 | 0.9154 |

Table 3B: Intercurrent Mortality Comparison Female Mice

| | Method | | | |
|---|---|---|---|---|
| | Cox | | Kruskal-Wallis | |
| | Statistics | P-Value | Statistics | P-Value |
| Time-Adjusted Trend Test | | | | |
| Depart from Trend | 0.3208 | 0.9561 | 0.4160 | 0.9369 |
| Dose-Mortality Trend | 0.0629 | 0.8019 | 0.1718 | 0.6785 |
| Homogeneity | 0.3838 | 0.9838 | 0.5878 | 0.9644 |

Table 4A: Tumor Incidence Rates (Male Mice) with *P*-values (Asymptotic Method) Less Than 0.05

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. in control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| BRAIN | LIPOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2081 > 0.025 |
| TESTIS | HAEMANGIOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2081 > 0.025 |
| KIDNEY | TUBULAR CELL ADENOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 2 | 0.0423 > 0.025 |
| EPIDIDYMIS | INTERSTITIAL CELL ADENOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2081 >0.025 |

Table 4B: Tumor Incidence Rates (Female Mice) with *P*-values (Asymptotic Method) Less Than 0.05

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. in control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| HAEMOPOIETIC SYSTEM | LEUKAEMIA, ERYTHROID [M] | Fatal | 0.00 | 0 | 0 | 0 | 0 | 2 | 0.0480 > 0.025 |
| SKIN AND SUBCUTIS | HISTIOCYTIC SARCOMA [M] | Fatal | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2090 > 0.025 |
| PITUITARY GLAND | ADENOMA, INTERMEDIATE LOBE [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 2 | 0.0658 > 0.025 |
| OVARY | CYSTADENOCARCINOMA [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2072 > 0.025 |
| UTERUS | STROMAL POLYP [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 2 | 0.0407 > 0.025 |
| MAMMARY GLAND | LIPOSARCOMA [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1961 > 0.025 |
| DUODENUM | ADENOCARCINOMA [M] | Fatal | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2162 > 0.025 |

Table 4C: Tumor Incidence Rates (Female Mice) with *P*-values (Asymptotic Method) Less Than 0.05 [a]

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. in control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| HAEMOPOIETIC SYSTEM | LEUKAEMIA, ERYTHROID [M] | Fatal | 0.00 | 0 | 0 | 0 | 0 | 2 | 0.0480 > 0.025 |
| SKIN AND SUBCUTIS | HISTIOCYTIC SARCOMA [M] | Fatal | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2090 > 0.025 |
| PITUITARY GLAND | [C]ADENOMA, ANTERIOR LOBE [B] | Incidental | 2.50 | 1 | 2 | 1 | 0 | 5 | 0.0400 > 0.005 |
| OVARY | CYSTADENOCARCINOMA [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2072 > 0.025 |
| MAMMARY GLAND | LIPOSARCOMA [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1961 > 0.025 |
| DUODENUM | ADENOCARCINOMA [M] | Fatal | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2162 > 0.025 |

[a] The following tumors were combined:

1. Under pituitary organ, adenoma intermediate lobe (B) and adenoma anterior lobe (B)
2. Under uterus organ, stromal polyp (B) and stromal sarcoma (M)

Table 5: Percentage of survival in the high dose group at the end of Weeks 52, 78, and 91

| | Percentage of survival | | | |
|---|---|---|---|---|
| Sex | End of 52 weeks | End of 78 weeks | End of 91 weeks | End of 103 weeks |
| Male | 95% | 83% | 70% | 60% |
| Female | 92% | 75% | 57% | 38% |

Table 6: Mean Body Weight (gms) for Male and Female Mice

| | Male Mice | | | Female Mice | | |
|---|---|---|---|---|---|---|
| Group | Day 0 of Study | End of Study | Weight Gain | Day 0 of Study | End of Study | Weight Gain |
| Control 1 | 31.7 | 47.8 | 16.1 | 23.8 | 42.2 | 18.4 |
| Control 2 | 31.5 | 46.6 | 15.1 | 23.7 | 41.2 | 17.5 |
| Control (Average) | 31.6 | 47.2 | 15.6 | 23.8 | 41.7 | 18.0 |
| Low | 32.0 | 47.5 | 15.5 | 24.5 | 40.4 | 15.9 |
| Medium | 31.7 | 45.8 | 14.1 | 24.4 | 40.1 | 15.7 |
| High | 31.7 | 41.7 | 10.0 | 24.3 | 34.8 | 10.5 |

Table 7: Percent Difference in Mean Body Weight Gain from Concurrent Controls

| Group | % of control Male | % of control Female |
|---|---|---|
| Low | 99.4% | 88.3% |
| Medium | 90.4% | 87.2% |
| High | 64.1% | 58.3% |

Table 8: Mortality Rate at the End of the Experiment

| | Control 1 | Control 2 | Low | Medium | High |
|---|---|---|---|---|---|
| Male | 47% | 42% | 42% | 42% | 40% |
| Female | 62% | 65% | 60% | 65% | 62% |

Table 9A: Intercurrent Mortality Rate Male Rats

| Week | Control 1 | | Control 2 | | 15 mg/kg/day | | 50 mg/kg/day | | 125mg/kg/day | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % |
| 0 – 52 | 2 | 3.3 | 1 | 1.7 | 1 | 1.7 | 1 | 1.7 | 2 | 3.3 |
| 53 – 78 | 9 | 18.3 | 2 | 5.0 | 5 | 10.0 | 10 | 18.3 | 6 | 13.3 |
| 79 – 91 | 7 | 30.0 | 5 | 13.3 | 17 | 38.3 | 6 | 28.3 | 6 | 23.3 |
| 92 – 103 | 14 | 53.3 | 12 | 33.3 | 12 | 58.3 | 8 | 41.7 | 12 | 43.3 |
| Terminal Sacrifice | 28 | 46.7 | 40 | 66.7 | 25 | 41.7 | 35 | 58.3 | 34 | 56.7 |

Table 9B: Intercurrent Mortality Rate Female Rats

| Week | Control 1 | | Control 2 | | 15 mg/kg/day | | 50 mg/kg/day | | 125mg/kg/day | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % | No. of Death | Cum % |
| 0 – 52 | 2 | 3.3 | 1 | 1.7 | 1 | 1.7 | 3 | 5.0 | 4 | 6.7 |
| 53 – 78 | 10 | 20.0 | 12 | 21.7 | 8 | 15.0 | 8 | 18.3 | 5 | 15.0 |
| 79 – 91 | 6 | 30.0 | 9 | 36.7 | 14 | 38.3 | 8 | 31.7 | 6 | 25.0 |
| 92 – 103 | 16 | 56.7 | 11 | 55.0 | 10 | 55.0 | 15 | 56.7 | 12 | 45.0 |
| Terminal Sacrifice | 26 | 43.3 | 27 | 45.0 | 27 | 45.0 | 26 | 43.3 | 33 | 55.0 |

Table 10A: Intercurrent Mortality Comparison Male Rats

| | Method | | | |
|---|---|---|---|---|
| | Cox | | Kruskal-Wallis | |
| | Statistics | P-Value | Statistics | P-Value |
| Time-Adjusted Trend Test | | | | |
| Depart from Trend | 9.6305 | 0.0220 | 9.5167 | 0.0232 |
| Dose-Mortality Trend | 0.1918 | 0.6615 | 0.1153 | 0.7342 |
| Homogeneity | 9.8223 | 0.0435 | 9.6320 | 0.0471 |

Table 10B: Intercurrent Mortality Comparison Female Rats

| | Method | | | |
|---|---|---|---|---|
| | Cox | | Kruskal-Wallis | |
| | Statistics | P-Value | Statistics | P-Value |
| Time-Adjusted Trend Test | | | | |
| Depart from Trend | 0.4222 | 0.9356 | 0.4090 | 0.9384 |
| Dose-Mortality Trend | 1.5079 | 0.2195 | 1.2162 | 0.2701 |
| Homogeneity | 1.9301 | 0.7486 | 1.6253 | 0.8042 |

Table 11A: Tumor Incidence Rates (Male Rats) with *P*-values (Asymptotic Method)
Less Than 0.05

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. in control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| PANCREAS (EXOCRINE) | HAEMANGIOSARCOMA [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2182 > 0.025 |
| HAEMOPOIETIC SYSTEM | LYMPHOMA, LYMPHOCYTIC [M] | Fatal | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2170 > 0.025 |
| LUNG | BRONCHIOLO-ALVEOLAR CARCINOMA | Incidental | 0.00 | 0 | 0 | 0 | 0 | 2 | 0.0307 > 0.025 |
| BRAIN | EPENDYMOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2099 > 0.025 |
| BRAIN | MALIGNANT RETICULOSIS [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2099 > 0.025 |

Table 11B: Tumor Incidence Rates (Male Rats) with *P*-values (Asymptotic Method)
Less Than 0.05 [a]

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. in control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| PANCREAS (EXOCRINE) | HAEMANGIOSARCOMA [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2182 > 0.025 |
| HAEMOPOIETIC SYSTEM | [C]LYMPHOMA [M] | Both fatal and incidental | 0.83 | 1 | 0 | 1 | 1 | 3 | 0.0462 > 0.025 |
| LUNG | BRONCHIOLO-ALVEOLAR CARCINOMA | Incidental | 0.00 | 0 | 0 | 0 | 0 | 2 | 0.0307 > 0.025 |
| BRAIN | [C]MALIGNANT ASTROCYTOMA [M] | Both fatal and incidental | 0.00 | 0 | 0 | 1 | 0 | 2 | 0.0586 > 0.025 |
| BRAIN | MALIGNANT RETICULOSIS [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2099 > 0.025 |

[a] The following tumors were combined:
1. Under Hematopoietic system, lymphoma (M) and lymphoma, lymphocytic (M)
2. Under Brain, astrocytoma (M) and ependymoma (M)

Table 11C: Tumor Incidence Rates (Female Rats) with *P*-values (Asymptotic Method)
Less Than 0.05

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. in control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| HAEMOPOIETIC SYSTEM | LYMPHOMA [M] | Fatal | 0.00 | 0 | 0 | 1 | 0 | 2 | 0.0587 > 0.025 |
| HAEMOPOIETIC SYSTEM | LYMPHOMA, LYMPHOCYTIC [M] | Fatal | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.2203 > 0.025 |
| LYMPH NODE (MESENTERIC) | HAEMANGIOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1935 > 0.025 |
| MAMMARY GLAND | CARCINOMA [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1875 > 0.025 |
| UTERUS | ADENOCARCINOMA [M] | Incidental | 0.83 | 1 | 0 | 0 | 0 | 2 | 0.0693 > 0.025 |
| HEART | SCHWANNOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1395 > 0.025 |

Table 11D: Tumor Incidence Rates (Female Rats) with *P*-values (Asymptotic Method) Less Than 0.05 [a]

i. Pooled Control Group

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. In control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| HAEMOPOIETIC SYSTEM | [C]LYMPHOMA [M] | Fatal | 0.00 | 0 | 0 | 1 | 0 | 3 | ***0.0163 < 0.025*** |
| LYMPH NODE (MESENTERIC) | HAEMANGIOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1935 > 0.025 |
| MAMMARY GLAND | CARCINOMA [M] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1875 > 0.025 |
| UTERUS | ADENOCARCINOMA [M] | Incidental | 0.83 | 1 | 0 | 0 | 0 | 2 | 0.0693 > 0.025 |
| HEART | SCHWANNOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1395 > 0.025 |

ii. Control Group 1

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. In control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| HAEMOPOIETIC SYSTEM | [C]LYMPHOMA [M] | Fatal | 0.00 | 0 | 0 | 1 | 0 | 3 | 0.0391 > 0.025 |
| HEART | SCHWANNOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1765 > 0.025 |

iii. Control Group 2

| Organ Name | Tumor Name | Overall tumor type | Tumor rate as PCT. In control group | CTR1 | CTR2 | LOW | MED | HIGH | P-Value (Exact Method) |
|---|---|---|---|---|---|---|---|---|---|
| HAEMOPOIETIC SYSTEM | [C]LYMPHOMA [M] | Fatal | 0.00 | 0 | 0 | 1 | 0 | 3 | 0.0396 > 0.025 |
| UTERUS | ADENOCARCINOMA [M] | Incidental | 0.83 | 1 | 0 | 0 | 0 | 2 | 0.0474 > 0.025 |
| HEART | SCHWANNOMA [B] | Incidental | 0.00 | 0 | 0 | 0 | 0 | 1 | 0.1622 > 0.025 |

[a] The following tumors were combined:

1. Under Hematopoietic system, lymphoma (M) and lymphoma, lymphocytic (M)

Table 12: Percentage of survival in the high dose group at the end of Weeks 52, 78, and 91

| Sex | Percentage of survival End of 52 weeks | End of 78 weeks | End of 91 weeks | End of 103 weeks |
|---|---|---|---|---|
| Male | 97% | 87% | 77% | 57% |
| Female | 93% | 85% | 75% | 55% |

Table 13: Mean Body Weight (gms) for Male and Female Rats

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Group | Day 0 of Study | End of Study | Weight Gain | Day 0 of Study | End of Study | Weight Gain |
| Control 1 | 184.3 | 771.7 | 587.4 | 138.7 | 479.9 | 341.2 |
| Control 2 | 184.1 | 776.6 | 592.5 | 138.2 | 474.8 | 336.6 |
| Control (Average) | 184.2 | 774.2 | 590.0 | 138.5 | 477.4 | 338.9 |
| Low | 183.8 | 763.0 | 579.2 | 139.4 | 468.9 | 329.5 |
| Medium | 187.5 | 738.5 | 551.0 | 137.9 | 394.1 | 256.2 |
| High | 183.2 | 610.2 | 427.0 | 142.1 | 361.9 | 219.8 |

Table 14: Percent Difference in Mean Body Weight Gain from Concurrent Controls

| Group | % of control Male | % of control Female |
|---|---|---|
| Low | 98.2% | 97.2% |
| Medium | 93.4% | 75.6% |
| High | 72.4% | 64.9% |

Table 15: Mortality Rate at the End of the Experiment

| | Control 1 | Control 2 | Low | Medium | High |
|---|---|---|---|---|---|
| Male | 53% | 33% | 58% | 42% | 43% |
| Female | 57% | 55% | 55% | 57% | 45% |

## Figures

Figure 1A: Kaplan-Meier Survival Curve for Male Mice

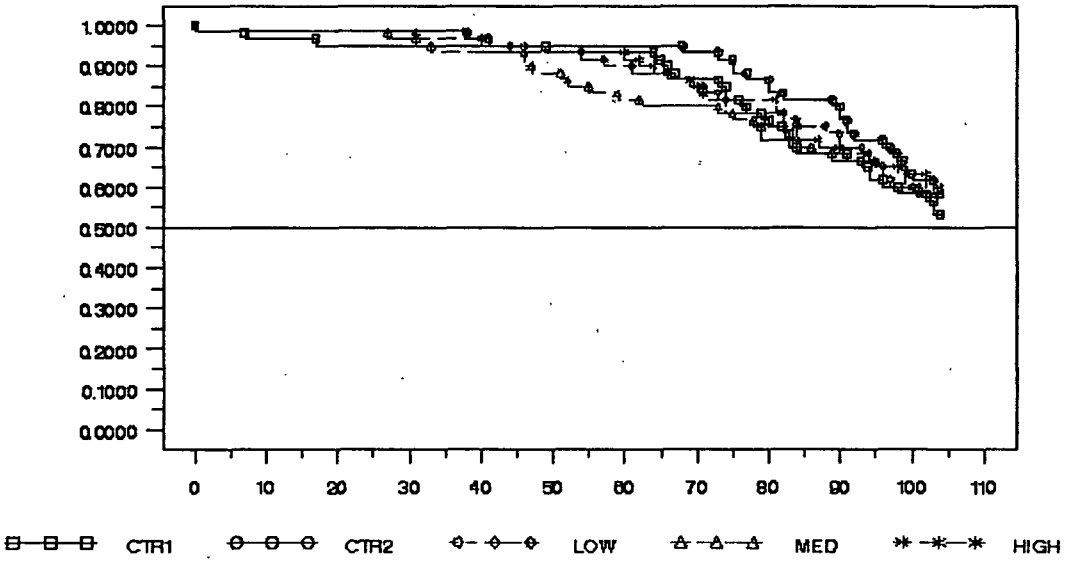



Figure 1B: Kaplan-Meier Survival Curve for Female Mice

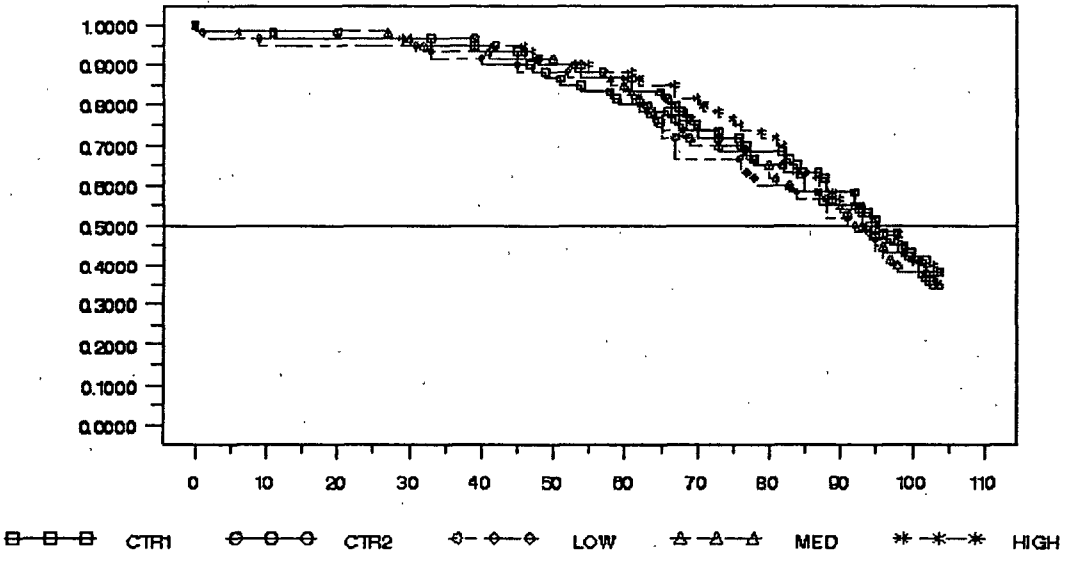


Figure 2A: Kaplan-Meier Survival Curve for Male Rats

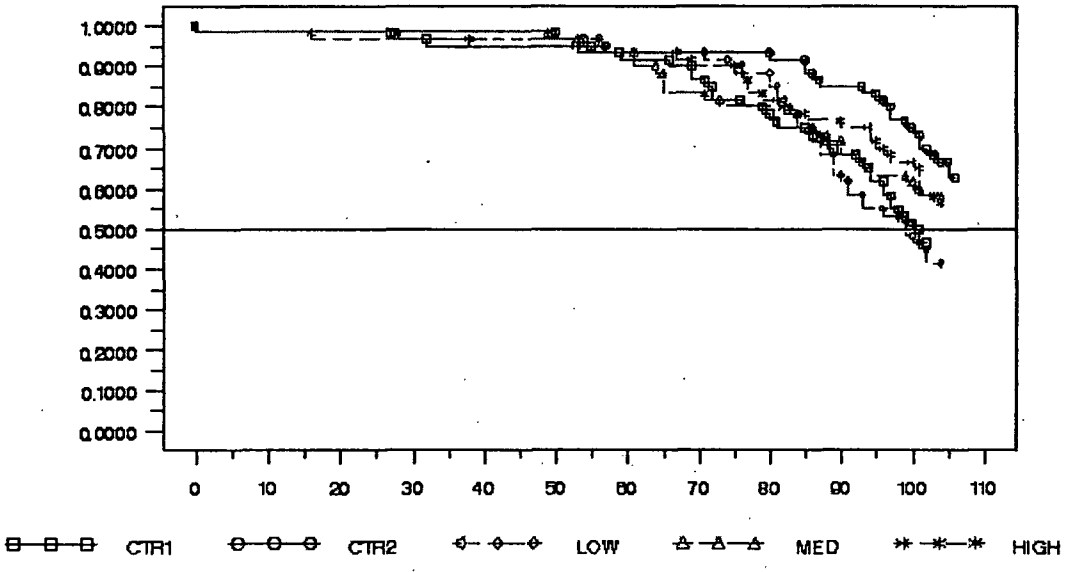
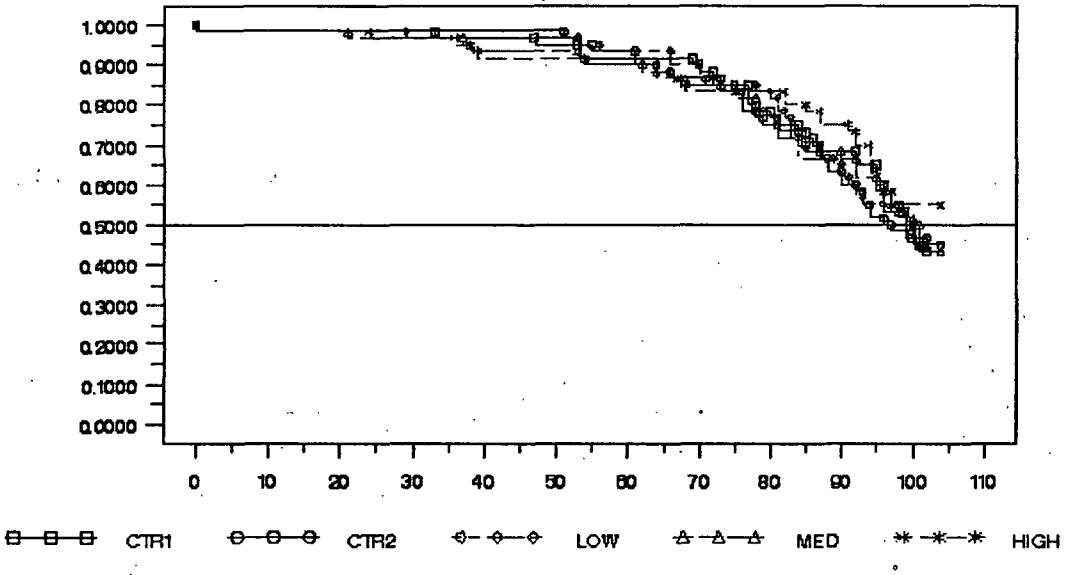



Figure 2B: Kaplan-Meier Survival Curve for Female Rats



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