Advisory Committee Briefing Document
Preclinical Pharmacology and Toxicology Summary

Drug: Pargluva® (muraglitazar, BMS-298,585)
Drug class: Peroxisome Proliferator-Activated Receptor dual agonist
Clinical Indication: Type 2 diabetes

Introduction

The peroxisome proliferator-activated receptors (PPAR) are nuclear receptors that regulate gene expression in response to ligand binding. Three PPAR receptor subtypes, namely gamma, alpha and delta (beta), have been identified. The development of PPAR agonist pharmaceuticals for the treatment of type 2 diabetes, dyslipidemia, and obesity is currently an area of great interest. The FDA has received data for more than 40 compounds including PPAR gamma, PPAR alpha, PPAR delta, PPAR dual (alpha/ gamma) or PPAR pan agonists. This extensive database permits identification of the toxicity profile specific to each PPAR receptor subtype. This summary will provide a brief overview of the toxicity profiles associated with exaggerated PPAR gamma or PPAR alpha activation for the class in general in addition to the preclinical toxicology data for muraglitazar.

The available clinical data for muraglitazar may address many of the potential safety concerns identified in the animal and clinical studies with muraglitazar or other PPAR agonists. The potential adverse effects of these agonists include fluid accumulation and edema leading to congestive heart failure, weight gain, hematologic effects, and liver, cardiac, skeletal muscle, renal, bone marrow, reproductive organ, and/or immune toxicity.

The fibrates, fenofibrate and gemfibrozil, are the approved drugs that mediate their effects on lipids via PPAR alpha activation. While the fibrates are well tolerated clinically, the compounds currently in development are 10 to 1000- fold more potent PPAR alpha agonists than the fibrates. Therefore, the long-term clinical safety experience with the fibrates may not predict the long-term safety profile for the more potent second generation PPAR alpha agonists. Similarly, the clinical safety experience with the approved PPAR gamma agonists, Actos (pioglitazone) and Avandia (rosiglitazone), may not predict the long-term safety profile for a dual agonist.

A major safety concern for the PPAR agonist drugs is the demonstrated carcinogenic potential in rodents. It has been known for sometime that many compounds, including the fibrates and pthalates, induce hepatocyte proliferation and liver cancer in rodents via PPAR alpha receptor activation. However, substantial scientific evidence suggests that primates are much less sensitive than rodents to the liver proliferative effects secondary to PPAR alpha activation. More recent data submitted to FDA from the two-year rodent carcinogenicity bioassays for 11 PPAR agonist pharmaceuticals have demonstrated drug-induced tumors in mice and rats of both sexes at multiple sites other than liver. According to EPA[1] and IARC[2] criteria, compounds that induce tumors in multiple species, in both sexes, and at multiple sites are classified as "probable human carcinogens". Since the tumors are observed in tissues with high PPAR receptor distribution and the mechanism of tumor induction for most tumor types is unknown, the human relevance of the

[1] EPA – US Environmental Protection Agency. (1986a) Guidelines for carcinogen risk assessment. Federal Register 51 (185) :33992
[2] IARC – WHO International Agency for Research on Cancer. Preamble to the IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. (www.cie.iarc.fr)

rodent tumors findings can not be ruled out. The absence of cancer findings in the available clinical safety database for the PPAR agonists pharmaceuticals, including muraglitazar, is not reassuring since most known human carcinogens require compound exposures or latency periods in excess of 10 years prior to the development of cancer.

Pharmacology

Muraglitazar is the first PPAR dual agonist (gamma/alpha) being reviewed by the FDA for marketing approval in the US. The rationale for the development of PPAR dual agonists is to simultaneously treat hyperglycemia and dyslipidemia via effects mediated by PPAR gamma and alpha activation, respectively.

While the mechanism by which PPAR gamma activation improves insulin sensitivity is not fully understood, PPAR gamma agonists are thought to improve glycemic control primarily via increased adipose proliferation and differentiation and increased glucose storage as fat. PPAR alpha agonists decrease triglycerides and increase HDL cholesterol with minimal effects on LDL cholesterol. PPAR alpha-dependent stimulation of lipoprotein lipase and apolipoprotein A-V expression are thought to increase the metabolism of triglycerides, while PPAR alpha-mediated induction of apolipoprotein A-1 and A-II are thought to mediate the increase in plasma HDL.

Most PPAR agonist compounds currently in development, including muraglitazar, do not have the thiazolidinedione structure of the previous PPAR gamma agonist drugs, Rezulin, Actos and Avandia. The toxicities observed with the various PPAR agonist subtypes are primarily receptor-mediated exaggerated pharmacological effects which result in toxicity. There is excellent cross-species concordance for most PPAR-mediated toxicities, i.e., adverse effects are observed in all species including humans. The dog appears to be particularly sensitive to some PPAR-induced toxicities, in particular liver toxicity, thymic atrophy, and bone marrow suppression. The sensitivity of rats and monkeys are more similar to humans for many PPAR- mediated toxicities and these animal models have been the most useful for predicting human adverse events and safe clinical exposures

PPAR Gamma Mediated Toxicities

PPAR gamma receptors are localized primarily in adipose tissue but also in the pancreas, vascular endothelium, bladder, colon, macrophages, and the immune system. Data from *in vitro* PPAR gamma transactivation studies (which estimate potency) for many drugs demonstrate that PPAR gamma activation potency for any given agonist is comparable across species.

Adipose: PPAR gamma-mediated adipose proliferation and differentiation are observed with all PPAR gamma agonists in all species (mouse, rat, dog, monkeys, and humans). This is observed as dose-related increased subcutaneous fat, and brown and white fat hyperplasia with macrovesiculation in brown fat and microvesiculation in white fat. Increased fatty infiltration of the bone marrow is observed with all compounds in all species, generally at drug exposures in the therapeutic range. The contribution of this fatty infiltration of the marrow to the mild anemia and less common bone defects observed with PPAR gamma and dual agonists is unclear. Fatty proliferation at multiple sites (subcutaneous, perirenal, periaortic, epididymal, subscapular) and fat deposition in other organs (pancreas, adrenal, thyroid/parathyroid, tongue) are also commonly observed. The subcutaneous adipose proliferation is observed as masses in short-term studies that progress to benign (lipomas) and malignant (liposarcomas) fat tumors in the 2- year rat carcinogenicity studies for several compounds (e.g., troglitazone, rosiglitazone, muraglitazar and others as shown in tables 2 and 3).

Fluid accumulation: Dose-dependent plasma volume expansion and extravascular fluid accumulation are observed with all PPAR gamma agonists in all species (mice, rats, rabbits, dogs, monkeys, humans). Dose and duration-dependent increases in the incidence of edema and cardiomegaly potentially leading to heart failure are observed in rats, dogs, monkeys, and humans. These effects were observed in animals and humans treated with muraglitazar. Clinical observations of dose-related excess edema and heart failure have lead to the termination of drug development programs or dose reductions in Phase 3 studies for some gamma and dual agonist compounds, including muraglitazar.

The relative contributions of fluid accumulation and adipose proliferation to PPAR gamma-mediated effects on weight gain are unclear. Atrial dilation and thrombi, ventricular hypertophy and dilation, pericardial/ thoracic/abdominal fluid accumulation and cardiac-related deaths are also commonly observed in the chronic toxicity studies in rats and monkeys treated with PPAR gamma and PPAR dual agonists.

Hematologic effects: The relative contributions of PPAR gamma mediated fluid accumulation and/or fatty infiltration of the bone marrow to the mild anemia observed in all species is unknown. Many PPAR alpha and dual agonists produce dose-related bone marrow suppression in rats, dogs and monkeys. Dogs appear to be the species most sensitive to this effect. Bone marrow suppression in rats and monkeys is observed less frequently than in dogs and generally only at high drug concentrations relative to therapeutic exposures. Dose-limiting neutropenia or thrombocytopenia has also been observed infrequently in humans treated with dual agonists (15% of compounds).
Muraglitazar produced bone marrow and lymphoid depletion in dogs treated for one month with doses ≥ 20 mg/kg/day (2 times clinical exposures). Chronic treatment with muraglitazar also produced bone marrow hypocellularity in monkeys at doses ≥ 2 mg/kg/day (8 – 14 times clinical exposures).

Immune toxicity: Thymic and splenic atrophy are commonly observed in animal species treated with PPAR gamma or dual agonists. Specific preclinical immunotoxicity studies have not been performed for most compounds.
Several deaths and moribund sacrifices secondary to systemic bacteremia occurred in rats treated with 300 mg/kg/day muraglitazar in the 6 month study (> 300 times the clinical dose). This led to an evaluation of lymphocyte subsets and antibody levels (IgG and IgM) for all dose levels. There were no clear effects on immunoglobulin levels with the 3 lowest doses of 0.3, 3 and 30 mg/kg/day (0.5, 5, and 50 times clinical exposures). Similarly decreases in lymphocyte subsets were only observed with the highest dose of 300 mg/kg/day. However, in male rats natural killer cell activity was significantly decreased with all doses (≥ 0.5 times clinical exposures).

Reproductive Effects: Delayed ovarian follicular development, decreased corpora lutea, and estrus cycle disruption in females and testicular tubular degeneration or atrophy in males have been observed in rats, dogs and monkeys treated with PPAR gamma or dual agonists.
Muraglitazar decreased corpora lutea in rats at ≥ 3 mg/kg (4-5 times clinical exposures) and increased testicular tubular degeneration/atrophy in dogs at ≥ 20 mg/kg/day (2 times clinical exposures), There were no drug-related reproductive findings in mice or monkeys treated with muraglitazar.

<u>PPAR Alpha Mediated Toxicities</u>
PPAR alpha receptors are located in the liver, kidneys, heart, diaphragm, skeletal muscle, esophagus, intestines, brown fat, and the immune system. Data from *in vitro* PPAR alpha transactivation studies for the compounds currently in development demonstrate that PPAR alpha

activation potency can differ significantly between rodents and primates. For the second generation PPAR alpha agonists, receptor activation potency is generally comparable in rodent species (mice ≈ rats) but less potent in rodents than primates (monkeys ≈ humans). This is also true for muraglitazar which is approximately 20 times less potent in rats than in humans (PPAR alpha EC 50 = 1.4 uM in humans, EC 50 > 32 uM in rats). Therefore, the estimation of safety margins for PPAR alpha mediated toxicities should consider potential species differences in pharmacodynamics rather than being based solely on pharmacokinetics (i.e., plasma drug AUC concentrations) as is the norm.

Liver: PPAR alpha is the receptor subtype responsible for inducing peroxisome proliferation (PP) in rodents, the observation resulting in the naming of the receptor class. PPAR alpha agonists cause peroxisome proliferation, hepatocyte proliferation, hepatomegaly, and liver cancer in rodents. The literature suggests that primates and humans are resistant to PPAR alpha-induced peroxisome proliferation and therefore, are not at risk for the development of liver cancer. Data submitted to the FDA for numerous PPAR agonists with potent alpha activity have demonstrated clear increases in peroxisome proliferation (2 to 5- fold) and hepatocyte proliferation (< 2-fold) in primates. However, the data are consistent with the conclusion that primates are less sensitive to the liver proliferative effects of PPAR alpha activation than are rodents. Mild increases in liver weight (25-30%) without evidence of PP were observed in monkeys treated with muraglitazar. In contrast to the clinical experience with Rezulin, serious liver toxicity has not been observed in the Phase 3 trials with other PPAR gamma or dual agonists, including muraglitazar.

Cardiac degeneration: Drug-related cardiac degeneration and/or necrosis have been observed in animals treated with several PPAR dual and many PPAR alpha agonists (> 50% of compounds). There was no evidence of direct cardiomopathy in mice, rats, or monkeys treated chronically with muraglitazar.

Myopathy: Skeletal muscle degeneration and/or necrosis are commonly observed in animals treated with agonists with PPAR alpha activity (> 50% of compounds). Rhabdomyolysis with acute renal failure has also been observed in Phase 1 clinical studies conducted with one PPAR dual and one PPAR alpha agonist. Skeletal muscle atrophy in dogs or degeneration in monkeys was observed infrequently with the highest doses of muraglitazar studied (> 25 times clinical exposures). However, evaluations of creatinine phosphokinase levels and multiple muscle sites were not performed as is recommended to adequately assess for this potential toxicity.
A specific 3-month rat study was performed with the co-administration of pravastatin and muraglitazar to assess the potential for additive or synergistic toxic effects in general, and for myopathy in particular. There was no evidence for additive myopathic effects of muraglitazar in combination with pravastatin. Several other rat toxicity studies evaluating co-administration of a PPAR dual or alpha agonist with atorvastatin have also failed to demonstrate additive myopathic effects.

Renal toxicity: Renal proximal tubular toxicity including vacuolation, dilation, degeneration and regeneration and even deaths secondary to renal failure have been observed in rats and monkeys treated chronically with some PPAR dual agonists (20% of compounds). An increased frequency of sarcomatous tumors of the renal tubules has also been observed in the 2-year rat carcinogenicity studies with some dual agonists. There were no changes in clinical chemistry or renal pathology indicative of renal toxicity in rats or monkeys treated chronically with muraglitazar.

Gastrointestinal Toxicity: Hyperkeratosis, erosions and ulcers of the stomach have been observed with several PPAR alpha agonists, most frequently in rodents. No evidence of these GI adverse effects were observed in mice, rats, or monkeys treated chronically with muraglitazar.

Table 1 - Summary of Toxicities and Safety Margins for Muraglitazar by Species

Species; Study duration Doses - mg/kg/day	NOAEL[3] Mg/kg	Safety Margin[4]	Lowest Adverse Effect Level and Toxic Effects Observed
Mouse, 3 months 4, 20, 100, 500 mg/kg	4	12 X	brown and white fat proliferation ≥ 20 mg/kg MTD* defined as 20 mg/kg based on 25% increase in heart weight at this dose (50 - 60 X)
Rat, 6 months 0, 0.3, 3, 30, 300 mg/kg	3	4 – 5 X	Adipose hyperplasia and vacuolation ≥ 30 mg/kg Cardiac hypertrophy (atrial and ventricular), thymic atrophy, inflammation of ear, nasal cavity, mammary gland, skeletal muscle, adipose at 300 mg/kg, but 300 mg/kg was lethal dose. (> 300 X)
Monkey , 9 and 12 months 0, 0.4, 2, and 5 mg/kg	0.4	2 – 3 X	Edema , increased liver/kidney weights, brown and white adipose proliferation ≥ 2 mg/kg (>12X)
Dog, 1 month 0, 0.2, 2, 20, 200 mg/kg	0.2	0.01 X (1 / 100 X)	QT prolongation, ↓ spleen wt ≥ 2 mg/kg (0.1X); ↑ BUN, ↓ RBC, ↑ ALT , adipose hyperplasia, bone marrow and lymphoid depletion , brain vacuolization ≥ 20 (2X)

* MTD = maximum tolerated dose for chronic rodent studies. Increases in heart weight > 25% at 3 months are associated with excess cardiac mortality in 2 year rodent carcinogenicity studies with PPAR gamma and dual agonists.

Overall Toxicology Conclusions:
PPAR gamma mediated adverse effects include adipose proliferation and deposition in tissues (e.g., bone marrow), weight gain, fluid accumulation resulting in mild anemia, edema, cardiomegaly, and increased cardiac-related mortality. Immune atrophy/depletion is also commonly observed. All of these toxicities were observed in the preclinical studies with muraglitazar. The no adverse effect doses (NOAEL) in animals provided adequate safety margins for the clinical doses in all species except dogs. Since dogs are known to be particularly sensitive to PPAR-mediated toxicities, monkeys were utilized for chronic safety assessments. In the *in vitro* PPAR gamma transactivation assays, which estimate drug concentrations required for receptor activation, muraglitazar has a human PPAR gamma potency comparable to rosiglitazone. Since muraglitazar will be administered in a comparable dose range as rosiglitazone, a comparable clinical safety profile to rosiglitazone might be expected for PPAR gamma-mediated toxicities.

The addition of PPAR alpha activity to the dual agonists confers the potential for many additional PPAR alpha-mediated toxicities. Therefore, the clinical experience with the approved gamma agonists may not be predictive of the clinical safety profile for a dual agonist. PPAR alpha mediated toxicities include peroxisome proliferation, liver hypertrophy and toxicity, skeletal and cardiac myopathy, bone marrow suppression, renal tubular and gastrointestinal (GI) toxicity. Liver toxicity, cardiomyopathy, renal tubular or gastrointestinal toxicity were observed infrequently such that no attribution to treatment could be concluded except at extremely high

[3] NOAEL – no adverse effect level – the dose without adverse effects.
[4] Safety margin based on drug exposures at NOAEL in animals / clinical drug exposures with the maximum human dose of 5 mg/ day.

doses relative to clinical exposures. Adverse effects on bone marrow and skeletal muscle were observed in dogs and monkeys treated with muraglitazar. The proposed clinical doses of muraglitazar approximate the minimum effective dose relative to human PPAR alpha activation and, therefore, should minimize the potential for exaggerated pharmacological effects which lead to toxicity.

The major safety concern for the PPAR agonist drugs is the demonstrated carcinogenic potential of these compounds in rodents and the absence of data regarding mode of action for most tumor types to permit an evaluation of the human relevance of the rodent tumor findings. The rodent carcinogenicity data for the drug class and muraglitazar are discussed in detail below.

Rodent Carcinogenicity Findings with the PPAR Agonists Drugs

The results of the 2-year mouse and rat carcinogenicity bioassays for 11 PPAR agonist drugs (5 gamma and 6 dual agonists) have been reviewed by the FDA. These studies are conducted by administering drug for the entire lifespan of the rodent (6 –104 weeks). Doses for evaluation are selected so that the low dose produces drug exposures in the therapeutic range and the high dose is the maximum tolerated dose (MTD) that doesn't produce significant toxicity.

The results of these studies demonstrate drug-related increases in a number of tumor types in mice and rats (see Tables 2 and 3 below). The sites of tumor development are consistent with the known distribution of PPAR receptors. The PPAR–induced tumor findings display the all of the characteristics defined by EPA[5] for significant rodent carcinogenicity findings. These include the following: 1) uncommon tumor types, 2) tumors at multiple sites, 3) tumors in multiple species, strains, and sexes, 4) progression from hyperplasia to benign to malignant tumors, 5) metastases and large proportion of malignant tumors, 6) unusual magnitude of tumor response, and 7) dose-related increases. None of the PPAR agonists are genotoxic in the standard ICH test battery.

Three sponsors have discontinued the clinical development of their dual agonist compounds when tumors were observed at all dose levels, including those producing drug exposures in the therapeutic range.

The well established triad of liver, pancreatic, and testicular cancer seen with PPAR alpha only agonists[6], such as fenofibrate and gemfibrozil, are not routinely seen with many dual agonists. The carcinogenicity data submitted to the FDA for the dual agonists are for agonists with potent gamma and relatively weaker alpha activity. Since the gamma-mediated effects on cadiomegaly are dose-limiting and establish the maximum dose evaluated in the rodent carcinogenicity studies, the alpha activity of the dual agonists is often not adequate to induce the prerequisite hepatomegaly and hyperplasia required for the induction of rodent liver tumors. This is true for muraglitazar which did not produce significant increases in liver weight or hepatocellular hyperplasia in mice or rats at the doses utilized in the two year carcinogenicity studies. (For muraglitazar, the rodent EC 50 for PPAR gamma = 60 -90 nM while the EC50 for PPAR alpha = 24,000 - 32,000 nM).

[5] EPA Guidelines for Carcinogen Risk Assessment. www.epa.gov/ncea/raf/cancer.htm

[6] PPAR-alpha agonist induced rodent tumors: modes of action and human relevance. Critical Reviews in Toxicology 33(6):655-780, 2003.

Table 2 - Tumor Findings with PPAR Gamma Agonists

Drug	Hemangiosarcoma	Bladder Tumor	Lipoma/ liposarcoma	Liver Tumor	Other Tumors
Troglitazone #	B6 Mice: M, F		Rat: M, F Wistar	Mice: F	
Rosiglitazone			SD Rat: M, F Lipomas only		
Pioglitazone		SD Rat: M bladder			Mice - cervix Leiomyosarcoma
A#	CD -1 Mice: M, F		Rat: M Wistar		Mice: M, F Gallbladder
B #	CD –1 Mice: M, F			Rat: F	Rat: M – stomach Leiomyosarcoma
C	CD-1 Mice, M. F				

discontinued for clinical safety reasons.

Table 3- Tumor Findings with PPAR Dual Agonists

Drug	Hemangiosarcoma	Bladder/Renal Tumor	Fibrosarcoma (skin)	Lipoma /Sarcoma	Liver Tumor	Other Tumors
D*	??	SD Rat: M, F Renal tubular		SD Rat: M	Rat: F	Rat –testicular, mammary, thyroid
E*	CD-1 Mice: M, F	SD Rat: M, F bladder/kidney	Rat: F		Mice:M, F	Rat, F–mammary
F*	CD-1 Mice: M, F	SD Rat: M, F bladder/kidney		Rat: M, F Mice: M, F		Mice: F – mammary Mice: M - stomach
G	Hamster: M		Wistar Rat:M, F		Rat: M, F	Rat: F - thyroid
Muraglitazar		SD Rat: M bladder		Rat: M, F		Mice: M gallbladder Rat: uterine
H	B6 Mice: M, F	F 344 Rat: M, F bladder/kidney				Rat: M, F - leukemia Rat: uterine

* - discontinued due to rodent tumor findings at all doses

In mice, both PPAR gamma and dual agonists increase the incidence of vascular tumors (hemangiosarcomas). Drug-induced increases in vascular tumors have been observed in CD-1 mice, B6C3F1 mice, and hamsters of both sexes with 8 of 12 compounds (4 gamma, 4 dual agonists). For most compounds, hemangiosarcomas are observed in multiple tissues consistent with the sites of spontaneous tumor formation (e.g., liver, spleen, skin, bone marrow). For one dual agonist compound, tumors were observed in adipose only. PPAR gamma receptors are known to be located in the vascular endothelium. For many of these drugs, vascular tumors were observed in mice at doses producing exposures in the therapeutic range (1-3 times clinical exposures).

Two mouse carcinogenicity studies were conducted with muraglitazar. In the first study, doses of 0, 0, 1, 5, and 20 mg/kg/day were administered to CD-1 mice for 2 years. In the second study, doses of 0 and 40 mg/kg/day were studied. Plasma drug exposures (AUC) attained with these doses in mice represent multiples of 3, 16, 60 and 140 times clinical exposures with 5 mg/day. Notably, statistically significant increases in drug-induced hemangiosarcomas were not observed in the 2-year mouse studies conducted with muraglitazar.

Drug-induced increases in lipomas and/or liposarcomas were observed in the 2-year rat carcinogenicity studies conducted with 3 gamma and 3 dual agonists. Tumors have been observed with increased incidence in both sexes of Sprague Dawley and Wistar rats. PPAR gamma receptors are highly distributed in adipose and PPAR activation results in adipocyte proliferation and differentiation. Therefore, it is not surprising that fat hyperplasia progresses to benign and malignant tumors with chronic dosing.

The rat carcinogenicity study conducted with muraglitazar evaluated doses of 0, 0, 1, 5, 30 and 50 mg/kg/day. Plasma drug exposures (AUC) attained with these doses in rats represent multiples of 1, 8, 35 and 50 times clinical exposures in diabetics receiving 5 mg/day. Increases in benign lipomas in female rats and malignant liposarcomas in male rats were observed only with the highest dose of muragiltazar tested (> 50X therapeutic exposures, see table 4).

Marked increased in transitional cell carcinomas of the urinary bladder have been observed in rats treated with 5/6 dual agonists and pioglitazone. The PPAR-induced tumors have been observed in Sprague Dawley and Fischer rats of both sexes. Muraglitazar produced bladder tumors in male rats at doses ≥ 5 mg/kg/day (≥ 8 times therapeutic exposures, see table 5). The low dose of 1 mg/kg that produced no bladder hyperplasia or carcinomas is associated with drug exposures roughly comparable to clinical exposures (1.3 X).

Table 4. Incidences of Subcutaneous Lipoma, Fibrolipoma, and Liposarcoma in Male and Female Rats treated with Muraglitazar

Group Number:	1	2	3	4	5	6
Dose (mg/kg/day):	0	0	1	5	30	50
Number Examined:	65	65	65	65	65	65
Subcutaneous Tissue (Males)						
Lipoma (B)	0	0	1	1	0	0
Fibrolipoma (B)	0	0	0	0	0	0
Liposarcoma (M)	1	0	1	1	1	7*
Lipoma(B)+Fibrolipoma(B)+Liposarcoma(M)	1	0	2	2	1	7*
Subcutaneous Tissue (Females)						
Lipoma (B)	0	0	0	0	1	2*
Fibrolipoma (B)	0	0	0	0	0	1
Liposarcoma (M)	1	1	1	1	2	1
Lipoma(B)+Fibrolipoma(B)+Liposarcoma(M)	1	1	1	1	3	4

B = benign; M = malignant
* Significant positive trend at p ≤ 0.025 level for a rare neoplasm (incidence < 1%).

Table 5. Incidences of Urinary Bladder Transitional Cell Carcinoma in Male Rats and Urinary Bladder Hyperplasia in Male and Female Rats treated with Muraglitazar

Group Number:	1	2	3	4	5	6
Dose (mg/kg/day):	0	0	1	5	30	50
Number Examined:	65	65	65	65	65	65
Urinary Bladder (Males)						
Transitional Cell Papilloma (B)	0	0	1	1	2	2
Transitional Cell Carcinoma (M)	2	3	2	8	24*	36*
Transitional Cell Papilloma(B)+Carcinoma(M)	2	3	3	9*	26*	38*
Hyperplasia, focal or diffuse, urothelium	5	4	4	8	8	8
Urinary Bladder (Females)						
Transitional Cell Papilloma (B)	0	0	0	0	0	0
Transitional Cell Carcinoma (M)	0	0	0	0	0	0
Hyperplasia, focal or diffuse, urothelium	1	0	0	2	4	8

B = benign; M = malignant
* Significant positive trend at p ≤ 0.025 level for a rare tumor (incidence < 1%).

The routine urinalyses performed in general toxicity studies are inadequate to evaluate the mode of action for the rat bladder tumors and, therefore, mechanistic data are not available for most compounds. PPAR agonists display minimal renal excretion (< 7%) so direct effects on the bladder epithelium from drug or metabolites in urine are unlikely. Extensive 3- and 21-month mechanistic studies in rats were performed with muraglitazar to elucidate the urinary compositional and bladder epithelial changes involved in the tumor etiology. These studies revealed multiple changes in the urine of male rats that provided a likely mode of action for muraglitazar-induced rodent tumors. Muraglitazar induced changes included:

1) Persistence of urine pH > 6.5 which favors formation of calcium and magnesium crystals and calculi in male rats on normal diets.
2) dose and time-dependent decreases in urine citrate , a chelator of calcium and magnesium and an inhibitor of crystal formation.
3) increased calcium and magnesium crystals in urine in association with proliferative mucosal responses.
4) bladder epithelial hyperplasia as determined by BrdU labeling after ≥ 3 months of treatment in males only– observed both dorsally and ventrally but more significant ventral effects consistent with ventrolateral deposition of irritating crystals.
5) Progression from mucosal hyperplasia at 3 months to nodular hyperplasia at 6 months to transitional cell tumors by 9 months in high dose male rats.
6) Bladder masses located primarily in the ventral and dome regions of the bladder consistent with cytotoxic and hyperplastic changes secondary to irritation by crystals.
7) Prevention of the formation of calcium and magnesium crystals and the consequent cytotoxic and proliferative responses in the bladder of male rats by acidification of the urine (via dietary supplementation with 1% ammonium chloride).

The results of the mechanistic studies support that the mode of action for bladder carcinogenesis in male rats treated with muraglitazar is via alterations in urine composition which predispose to crystal formation, bladder irritation, and consequent epithelial proliferation.

While the mechanistic data implicating a male rat specific mode of action for muraglitazar induced bladder tumors is convincing, the bladder findings for the class still raise some concern regarding the potential clinical relevance of the rat bladder tumor findings.
For most dual agonist compounds, the bladder tumors have been observed in both sexes of rats not just in males and in the renal pelvis as well as the bladder.
Bladder hyperplasia has been observed in the chronic toxicity studies in dogs and monkeys with several other dual agonists which brings into question whether this is truly a rat-specific effect. While bladder hyperplasia was not observed in the 9 or 12 month monkey studies with muraglitazar, staining for epithelial proliferation (an early predictive change) was not performed. Chronic dog and monkey studies conducted with PPAR agonists are not of sufficient duration or sample size to permit the observation of drug-induced tumors, so the absence of tumor findings in these studies is not reassuring. In addition, several PPAR gamma and dual agonists have tested positive as bladder tumor promoters in the butyl, hydroxybutyl- nitrosamine (BBN) initiation-promotion model[7]. In BBN-initiated rats treated with PPAR agonists, marked increases in the incidence of bladder tumors were observed within 6 months in the absence of demonstrable crystalluria.

Other sarcomatous tumors in mesenchymally derived tissues observed less frequently in the carcinogenicity study with PPAR agonists include fibrosarcomas of the skin, leiomyosarcoma

[7] Lubet et al. Proceedings of the American Association for Cancer Research, 45 Abstract 3132, March 2004. Data for other compounds testing positive in this model is proprietary.

(muscle tumors) of the stomach or cervix, and sarcomatous tumors of the renal tubules. Gallbladder adenomas in mice and uterine or mammary adenocarcinomas in rats have also been observed with some compounds. In the carcinogenicity studies for muraglitazar, increases in uterine tumors in rats and gallbladder adenomas in mice were observed only with the highest doses tested (> 50-60 times therapeutic exposures). These data for these tumor types are summarized in tables 6 and 7.

Table 6. Incidences of Uterine Adenoma and Carcinoma in Muraglitazar Treated Rats

Group Number:	1	2	3	4	5	6
Dose (mg/kg/day):	0	0	1	5	30	50
Number Examined:	65	65	65	65	65	65
Uterus						
Squamous Cell Papilloma (B)	1	0	1	0	0	1
Squamous Cell Carcinoma (M)	0	0	0	3	1	3[a]
Squamous Cell Papilloma (B) + Squamous Cell Carcinoma (M) [b]	1	0	1	3	1	4[a]
Adenoma (B)	0	1	0	1	2	1
Carcinoma (M)	2	4	6	1	5	6
Adenoma (B) + Carcinoma (M) [b]	2	5	6	2	7	7[a]
Squamous Cell Papilloma(B) + Squamous Cell Carcinoma(M) + Adenoma(B) + Carcinoma(M) [b]	3	5	7	5	8	11[a]
Metaplasia, squamous, endometrium [c]	18	14	20	15	21	11

B = benign; M = malignant

[a] Not a significant positive trend (Peto analysis) for a common neoplasm at the $p \leq 0.005$ level.

[b] Since these neoplasms are derived from uterine endometrium, it was considered biological relevant to statistically analyze all of the above combinations.

[c] A non-neoplastic precursor change for squamous cell papilloma and carcinoma.

The sponsor considered the gallbladder hyperplasia and adenomas in males to be drug-related since the incidence of mucosal epithelial hyperplasia and adenoma was greater than the historical mean or range. The control incidence of hyperplasia in male mice is 2/370 (0.54%) and the control incidence of gallbladder adenoma in male mice is 1/370 (0.27%). No proliferative changes were noted in the biliary tree of rats or the gallbladders of dogs or monkeys.

Table 7. Incidences of Gallbladder Hyperplasia and Adenoma in Muraglitazar Treated Mice

Doses, mg/kg/day	**0**	**0**	**1**	**5**	**20**	**40**
Gallbladder hyperplasia - males	1	0	5	9	6	6
Gall bladder adenoma (B) - males	0	0	0	0	1	2
Gallbladder hyperplasia - females	0	1	0	4	3	4

Conclusions regarding Rodent Carcinogenicity Findings with PPAR Agonists

Evidence from completed carcinogenicity studies with 11 PPAR compounds (5 gamma, 6 dual agonists) demonstrate that PPAR agonist are rodent carcinogens. Since PPAR- induced tumors are observed in both sexes of multiple species and strains and at multiple sites, these compounds are classified as “probable human carcinogens”. Since the tumors are observed at sites of high

PPAR receptor distribution and appear to be secondary to PPAR activation, human relevance can not be ruled out.

While for many PPAR agonists the tumors are observed at all dose levels and with drug exposures in the therapeutic range, the data for muragitazar do not represent as compelling a concern with regards to carcinogenic risk. While the urinary bladder tumors were observed at relatively low multiples of clinical exposures ($\geq$ 8X), the mode of action data provide evidence that the tumors observed in male rats are due to urolithiasis secondary to PPAR induced changes urinary pH, citrate, and calcium concentrations. The absence of evidence of bladder hyperplasia in monkeys treated with muraglitazar for 9 or 12 months is also reassuring.

The liposarcomas and uterine tumors in rats and gallbladder tumors in mice displayed only modest increases in frequency at the highest doses tested (> 50-60 times clinical exposures). Since PPAR agonist drugs are not genotoxic, the carcinogenicity appears to be mediated by exaggerated pharmacologic effects observed as tissue hyperplasia progressing to benign and malignant tumors. Since thresholds can be established for compounds producing tumors via epigenetic (non-genotoxic) mechanisms, the 50-fold safety margins for muraglitazar-induced tumors suggest a negligible human cancer risk.

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