



U.S. Food and Drug Administration

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The Regulatory Challenges of Drug-induced Phospholipidosis

ACPS meeting, APRIL 14, 2010
Introduction: Nakissa Sadrieh, Ph.D.

Outline of this session

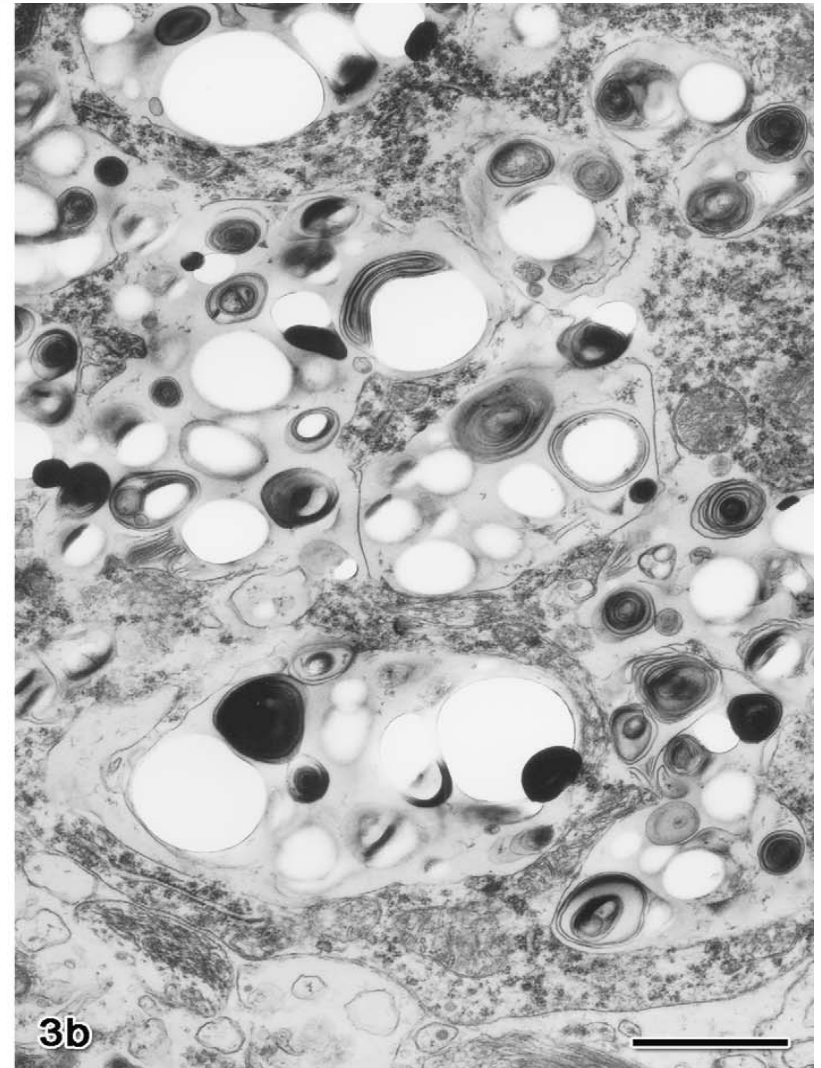
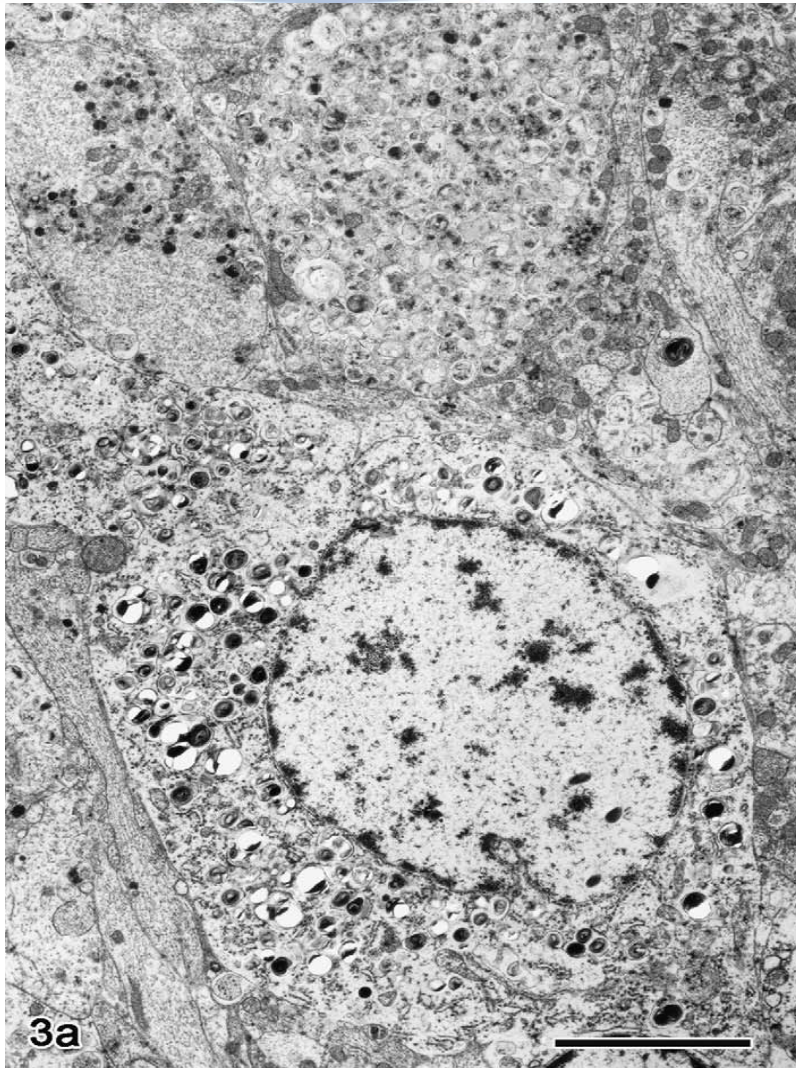
- **Introduction (Nakissa Sadrieh)**
- **The biology of phospholipidosis (Mark Reasor)**
- **Development of phospholipidosis biomarkers (Frank Hsieh)**
- **Accomplishments of the phospholipidosis working group (Jim Willard)**
- **Industry perspective (Kenneth Hastings)**
- **Questions for the advisory committee members**

Drug-induced Phospholipidosis (PLD)

- Excessive accumulation of phospholipid and drug within cells.
- PLD has been observed in various tissue types including lung, liver, kidney, heart, eye, and brain.
- PLD is recurrent pathological feature in toxicity studies, more commonly in animals, but also in humans.
- Labels include PLD findings during non-clinical study and indicate that the significance of PLD findings in human is unknown.

Histopathology of PLD

- By light microscopy, PLD can be identified by the appearance of foamy macrophages or cytoplasmic vacuoles in many cell types.
- By Transmission Electron Microscopy (TEM) (gold standard for identifying PLD), membranous lamellar inclusions, concentric multilamellar bodies, myeloid bodies, and other similar structures can be observed.

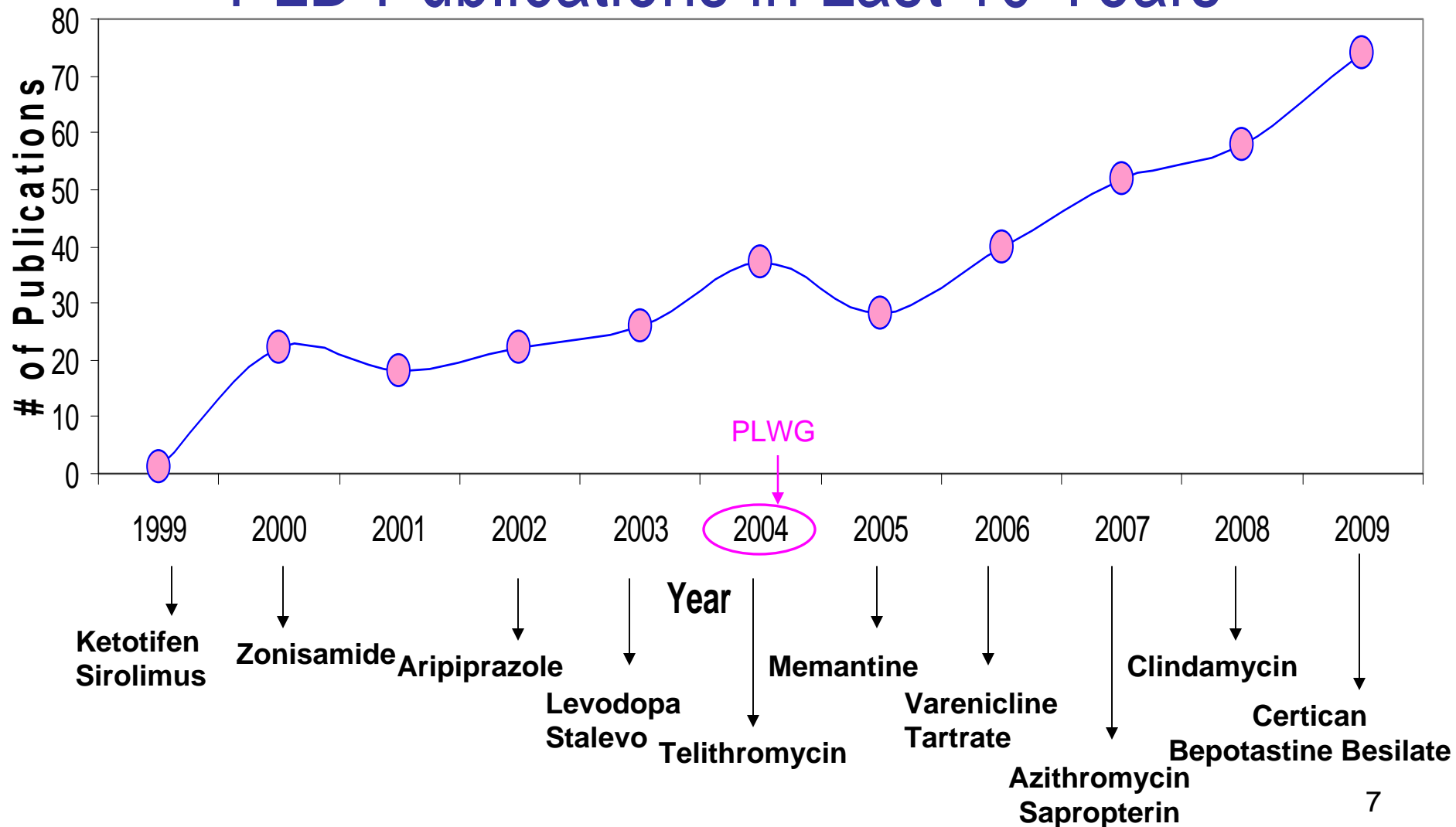


Cartwright et al., Toxicologic Pathology, (2009) 7, 902-910

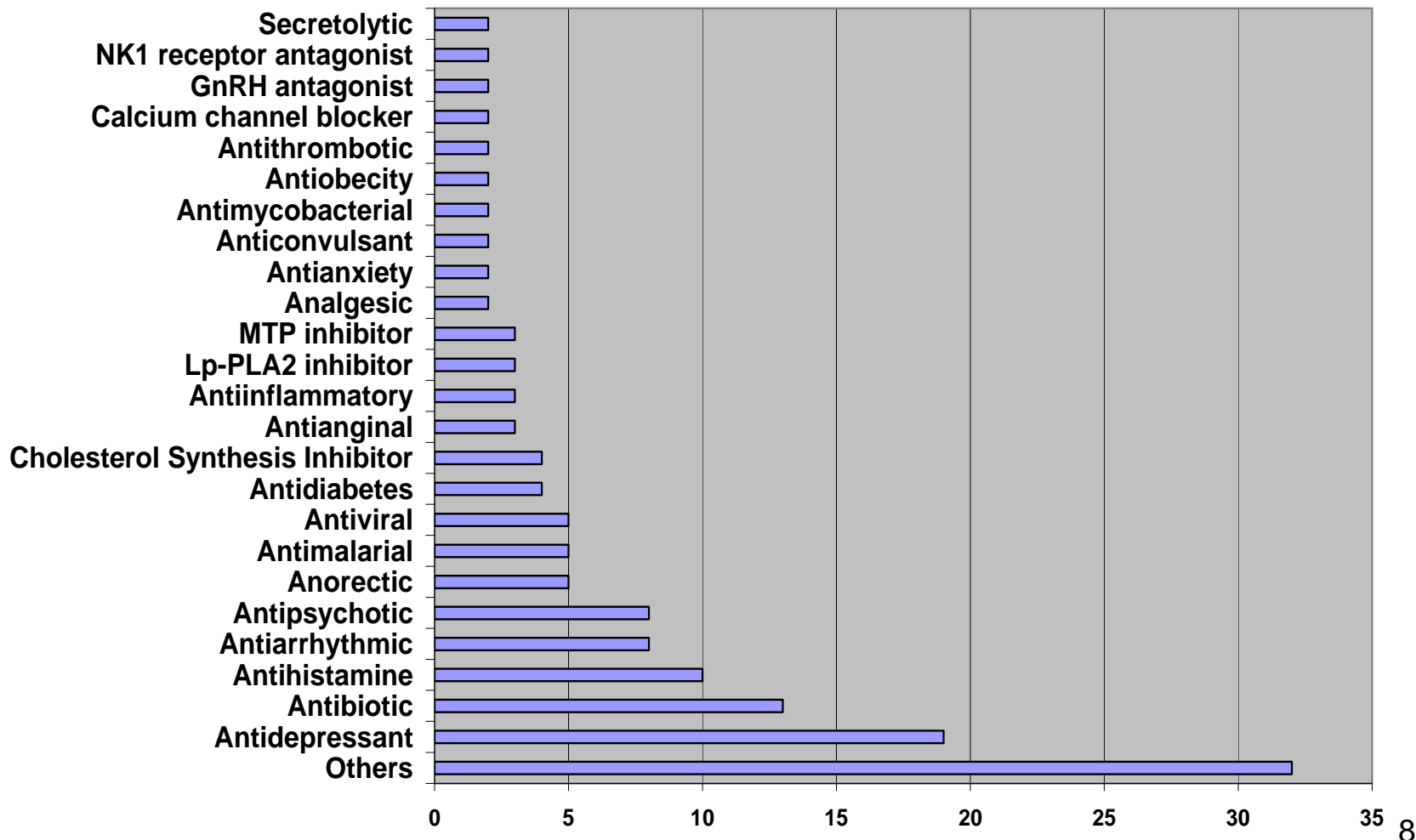
History

- In 1948, Nelson and Fitzhugh showed that
 - 2-year administration of chloroquine to rats induced foamy macrophages.
 - The phenomenon was called ‘foam cell syndrome’.
- Since 1970, when the term phospholipidosis started to be used, 532 publications have reported PLD findings.

PLD Publications in Last 10 Years



Pharmacological classes of PLD-inducing drugs



Why do only a small number of phospholipidosis-inducing drugs show toxicity ?

- 1. Phospholipidosis is an adaptive response by the host in response to the presence of a drug, rather than a toxic manifestation.**
- 2. Phospholipidosis that is seen in animals is not predictive of similar findings clinically.**

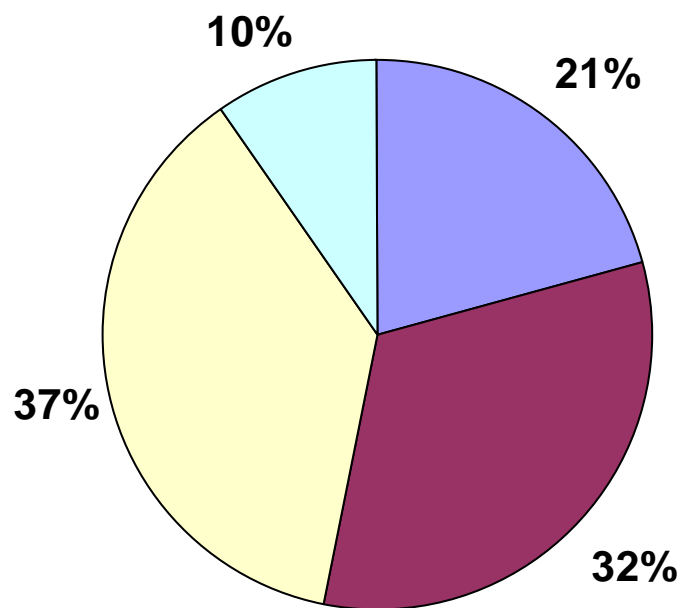
What have we learned about PLD-inducing drugs?

- Associated with every pharmacological class.
- Not always associated with toxicity in preclinical studies.
- No direct association with clinical outcome regarding structural pathology or functional toxicity.
- Most of PLD inducing drugs are cationic amphiphilic drugs (CADs).

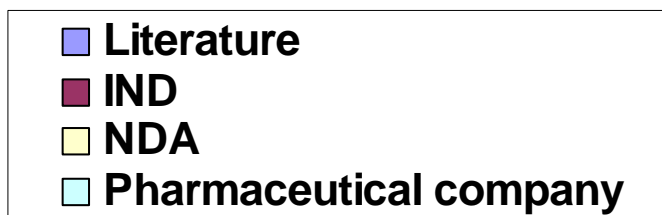
How many drugs are associated with PL?

- From the literature, over 50 marketed and experimental drugs containing Cationic Amphiphilic Drug (CAD) structure have been reported to induce phospholipidosis in vitro or in vivo.

FDA database contains 385 PLD positive compounds



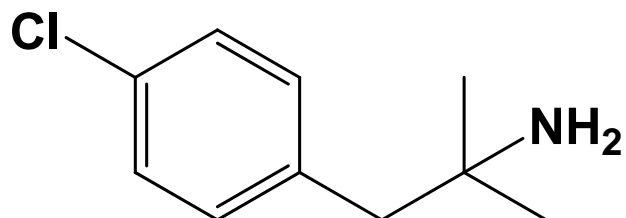
Total: 385 PLD Positive compounds
NDA: 143
IND: 124
Literature: 81
Pharmaceutical Company: 37



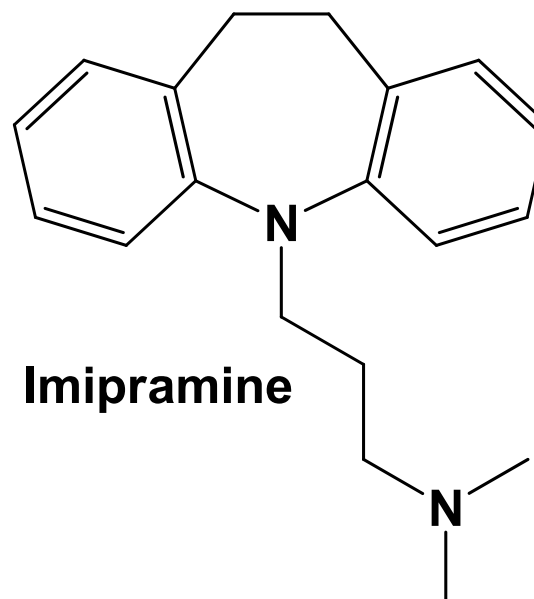
Cationic Amphiphilic Drugs (CADs)

- Contain hydrophobic ring structure and hydrophilic side chain with cationic amine group. At physiological pH and acidic milieu, the basic amine groups tend to be protonated.
- More than 290 PLD-inducing compounds in FDA database are CADs.
- Based on the CAD's structural property, PLD can be predicted by in silico methods.

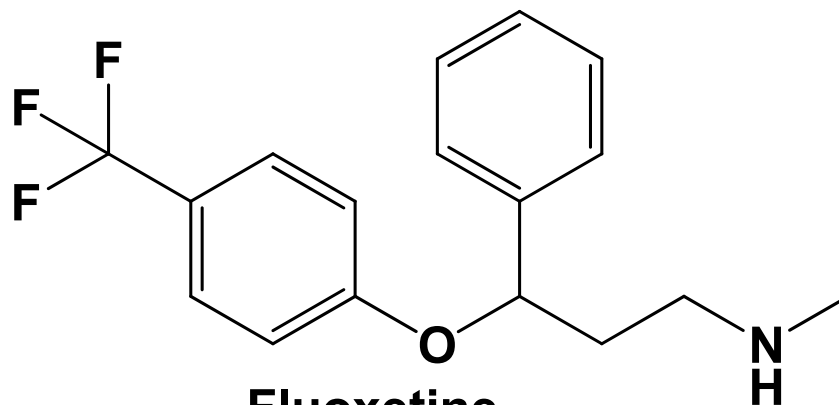
Examples of CADs



Chlorphentermine



Imipramine



Fluoxetine

What does industry do when a drug candidate causes PLD?

- Sponsors have developed risk management approaches to screen out PLD-inducing drugs.
- Because there has not been an official FDA policy on PLD, drugs that exhibit PLD have been dealt with on a case-by-case basis, by industry and FDA.
- Considerations on developing a drug that causes PLD can have included:
 - Risk benefit considerations
 - Target population
 - Indication
- Sponsors have chosen to terminate the development of a candidate drug (sometimes good drugs), when PLD was seen in certain organs.

Issues to be addressed

- How does CDER make the best regulatory decisions in the presence of PLD findings?
- How can CDER provide sponsors with the best advice during drug development?
- What does industry expect from CDER with respect to advice regarding the development of PLD-inducing drugs?

To address the issues

- Phospholipidosis Working Group (PLWG) created in response to the Critical Path Initiative (2004).
- PLWG goal:
 - To assess whether there should be clinical concern in the presence of PLD findings in animals.
 - To develop better tools to predict if drugs could induce PLD.
 - To investigate if a non-invasive biomarker for phospholipidosis could be developed, in order to detect phospholipidosis in patients treated with these phospholipidosis-inducing drugs.
 - To help industry by developing a guidance document,

Questions for the Committee

- As we consider preparing guidance regarding phospholipidosis:
 - a. Are the current CDER activities focused on the appropriate scientific and regulatory issues/concerns?
 - b. Are there additional issues we should be focusing on?

OVERVIEW OF DRUG-INDUCED PHOSPHOLIPIDOSIS

Mark J. Reasor, Ph.D., D.A.B.T.

Department of Physiology and Pharmacology
Robert C. Byrd Health Sciences Center
West Virginia University
Morgantown, WV
(mreasor@hsc.wvu.edu)

Overview

- Features
- Historical perspective
- Significance
- Characteristics
- Mechanisms of induction
- Toxicological significance

What is Drug-Induced Phospholipidosis?

Distinguishing Features

- 1) There is excessive accumulation of one or more polar phospholipids in cells.
- 2) There is the appearance of membrane-bound cytosolic inclusions with a lamellar or crystalloid structure. The inclusions are primarily lysosomal in origin (*and termed lamellar bodies/inclusions, multilamellated myelin figures, myelinoid bodies, concentric lamellar bodies, etc.*). **Observation requires TEM and is the hallmark morphologic feature and gold standard for assessment.**
- 3) The inducing drug accumulates in association with the excess phospholipid.
- 4) The alterations generally demonstrate reversibility after discontinuance of drug treatment.

History of Studies on Drug-Induced Phospholipidosis

Notable Publications

- 1948 Nelson & Fitzhugh described the occurrence of foamy macrophages in several tissues of rats fed chloroquine for 2 years. (*Arch. Pathol.* 45:454-462, 1948)
- 1968 Gleiser et al., observed myelinoid bodies in the CNS of pigs treated with chloroquine. (*Am. J. Pathol.* 53(1):27-45, 1968)
- 1970 Franken et al., reported the presence of huge foam cells in the alveoli of rats fed chlorphentermine for 6 weeks. This study appears to have triggered interest in this condition. (*Arzneimittelforschung* 20(3):417, 1970)
- 1970 Japanese investigators reported a fatty liver condition with lamellar bodies in patients treated with diethylaminoethoxyhexestrol (*Jpn J. Exper. Med.* 40:127-140, 1970)
- 1975 Lüllmann et al., published the first comprehensive review on drug-induced phospholipidosis. (*Crit. Rev. Toxicol.* 4:185-218, 1975)

Early pioneers in drug-induced phospholipidosis were Heinz Lüllmann and Renate Lüllmann-Rauch from the University of Kiel in Germany

Pub Med citations – key word “phospholipidosis”

1960 - 1969 = 0
1970 - 1979 = 35
1980 - 1989 = 116
1990 - 1999 = 76
2000 - 2009 = 101

Why is Drug-Induced Phospholipidosis a Potentially Serious Concern?

- Phospholipidosis may appear in the pre-clinical phase of drug development in one or more animal species resulting in concern about its potential to occur in clinical trials.
- Because of the pathological appearance of tissues/cells, which has similarities to certain inborn errors of metabolism, e.g., Niemann-Pick type-C Disease, the U.S.F.D.A. and corresponding regulatory agencies in Europe and Japan have generally considered phospholipidosis a potentially adverse response and are hesitant to approve drugs that induce this condition. Additionally, the lack of information about the consequences of the presence of phospholipidosis in cells/tissues adds to the problem.
- Consequently, drug development may be halted while the sponsor is required to answer questions or conduct additional studies posed by the agencies, thus adding considerable time and expense to the cost of development. On many occasions, drug development has been terminated because of the presence of phospholipidosis.

Drug-Induced Phospholipidosis

Results From Exposure to Cationic, Amphiphilic Drugs (CADs)

CADs are characterized by:

- 1) a hydrophobic domain consisting of an aromatic/and or aliphatic ring structure which may be substituted with one or more halogen moieties
- and
- 2) a hydrophilic domain containing one or more primary or substituted nitrogen groups, some of which are protonated at physiological pH or become protonated within the lysosome.

Representative Cationic, Amphiphilic Drugs (CADs) That Induce Phospholipidosis (Class/Drug)

Anorectic

Chlorphentermine
Cloforex
Fenfluramine

Antiarrhythmic

Amiodarone*
Perhexiline*

Antidepressant

1-chloroamitryptiline
Imipramine
Maprotiline
Zimelidine

Antihistaminic

Chlorcyclizine
Meclizine
Norchlorcyclizine
Hydroxyzine

Antimalarial

Chloroquine*

Mepacrine

Antianginal

4,4'-diethylamino-
ethoxyhexestrol*

Antiestrogen

Tamoxifen

Antithrombotic

RMI 10.393

Neuroleptic

Clozapine

SSRI

Fluoxetine*
Citalopram

Cholesterol Synthesis

Inhibitor

AY-9944
Boxidine
Triparanol

Antibiotic

Erythromycin
Gentamicin*
Azithromycin

Schistosomicidal

IA-3

Secretolytic

Ambroxol
Bromhexine

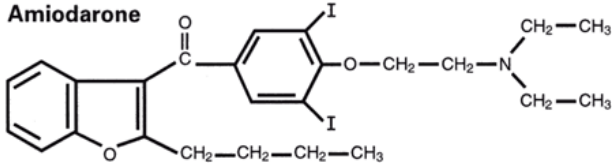
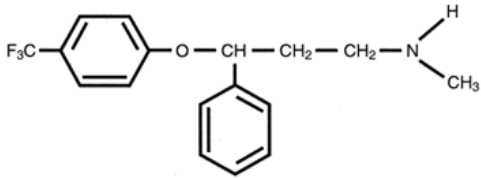
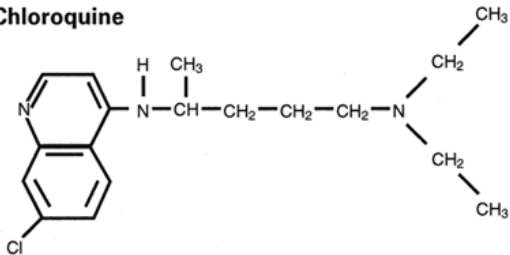
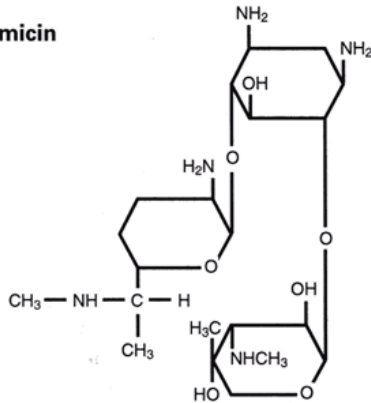
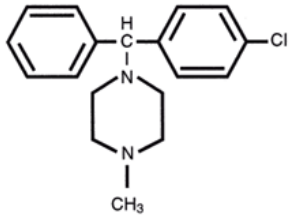
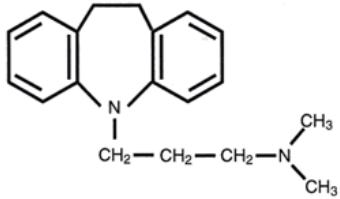
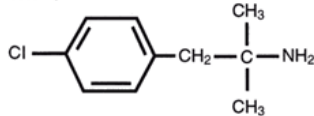
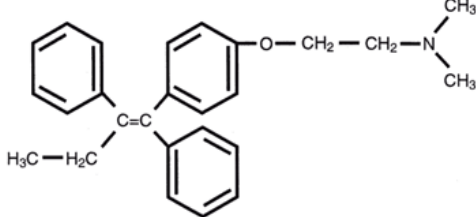
Tranquilizer

AC-3579

* Humans

The induction of phospholipidosis is not a function of the pharmacologic action of the drug.

Structures of Representative Cationic Amphiphilic Drugs That Induce Phospholipidosis

<p>Amiodarone</p> 	<p>Fluoxetine</p> 
<p>Chloroquine</p> 	<p>Gentamicin</p> 
<p>Chlorcyclizine</p> 	<p>Imipramine</p> 
<p>Chlorphentermine</p> 	<p>Tamoxifen</p> 

Characteristics of Drug-Induced Phospholipidosis

- Phospholipidosis is induced in many tissues in the body, and in cell culture, in generally a time-and dose (concentration)-dependent manner.
- No species, gender, or age group appears to be excluded from susceptibility to induction of phospholipidosis.
- The site(s) of induction may vary among and within species and cells/tissues and is(are) not predictable based upon the structure of a drug. For example, a drug may induce phospholipidosis in certain tissues in rats but not dogs or in the lungs of one strain of rats but not another.

Characteristics of Drug-Induced Phospholipidosis

- Many tissues are targets for the induction of phospholipidosis. The lungs are a principal target tissue with alveolar macrophages being very susceptible becoming enlarged, filled with phospholipid, and developing a “foamy” appearance under light microscopy. The liver is a target tissue for many drugs. In affected tissues/cells, most, if not all, classes of phospholipids will be increased.
- CADs that are effective in inducing phospholipidosis in cell culture may not do so with *in vivo* administration.

As a result of these factors, it is not possible to predict in what species, tissues or cells a CAD will induce phospholipidosis, if at all.

Characteristics of Drug-Induced Phospholipidosis

- CADs may accumulate in phospholipidotic tissue to a many-fold higher level compared to the plasma level.
- The rate of reversibility of phospholipidosis varies among drugs, tissues and species, and may occur minimally if at all. It is related to the dissociation rate of the drug from the bound phospholipid and elimination from the tissue.

Factors Influencing Development of Drug-Induced Phospholipidosis

A number of factors can influence whether a CAD will cause phospholipidosis *in vivo*.

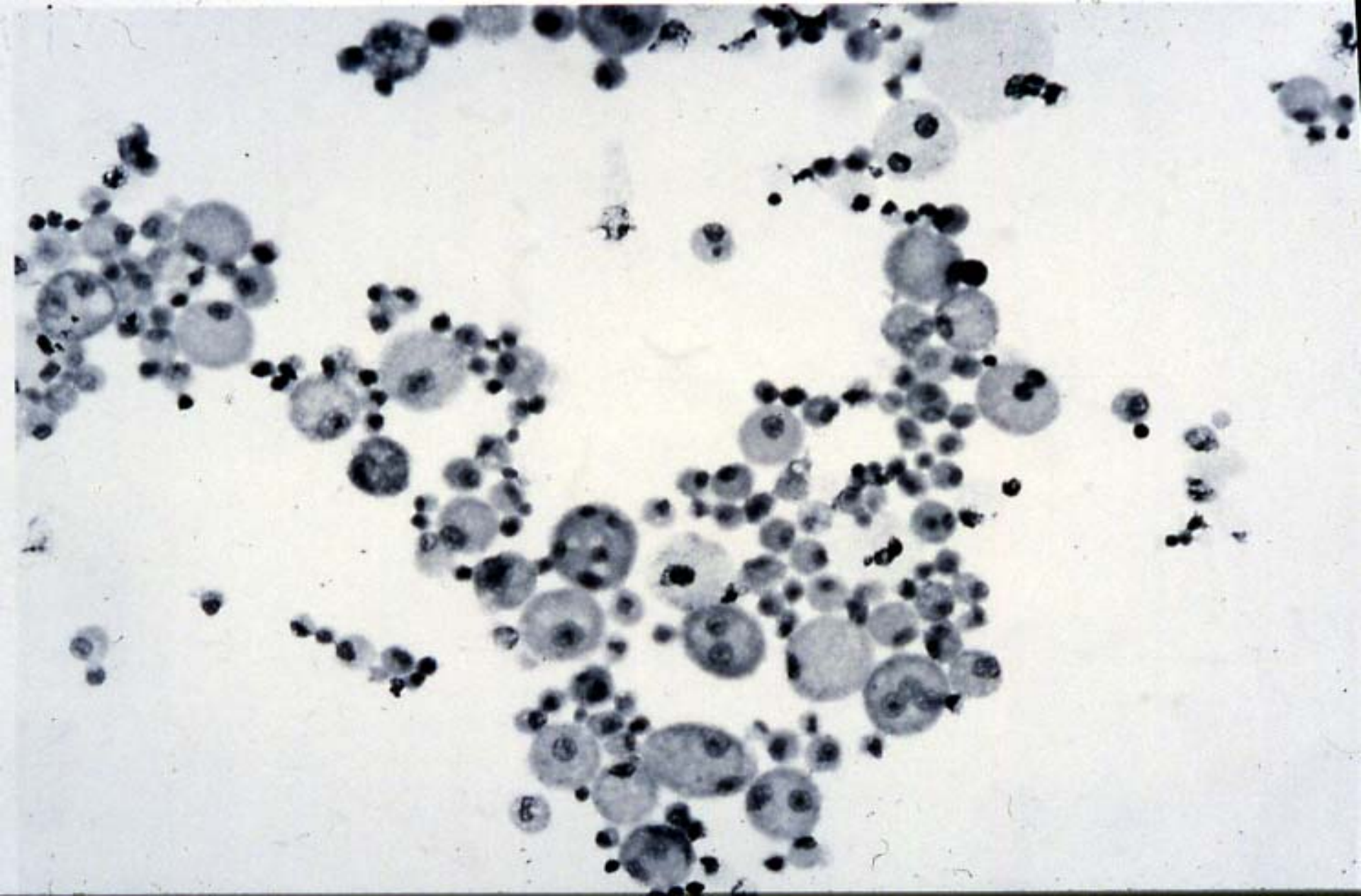
1) Metabolism of the drug

- a. Rapid metabolism may prevent induction of phospholipidosis *in vivo*.
- b. Metabolism may result in a metabolite that is effective in inducing phospholipidosis

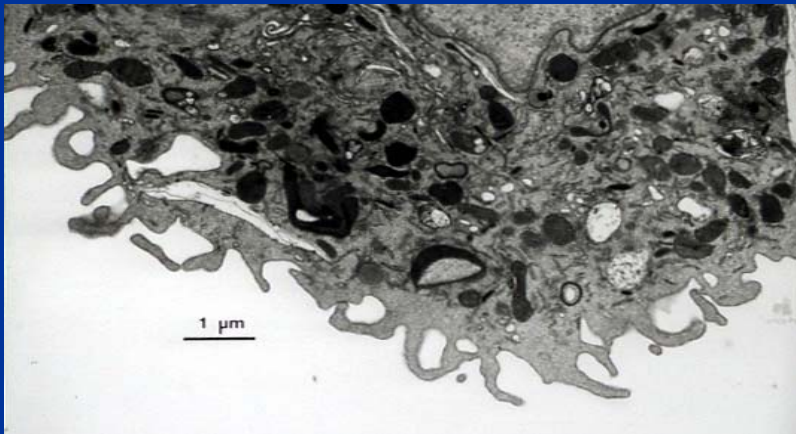
2) Tissue distribution of the drug/metabolites

3) Phospholipid level and classes in the tissue

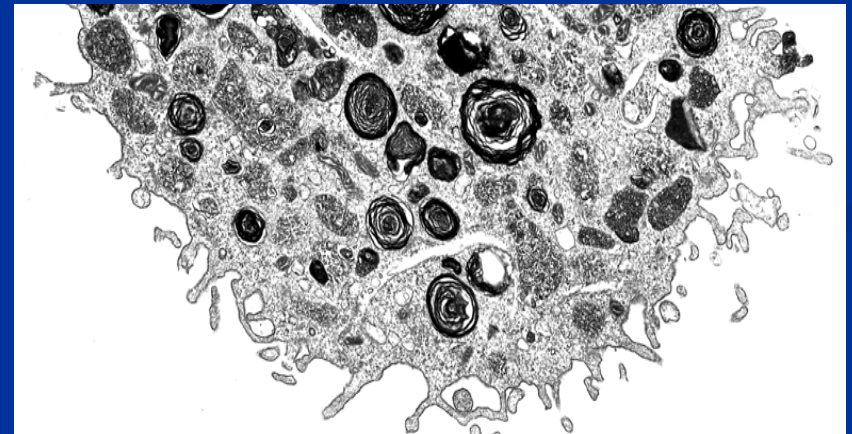
4) Binding affinity of the drug to phospholipids



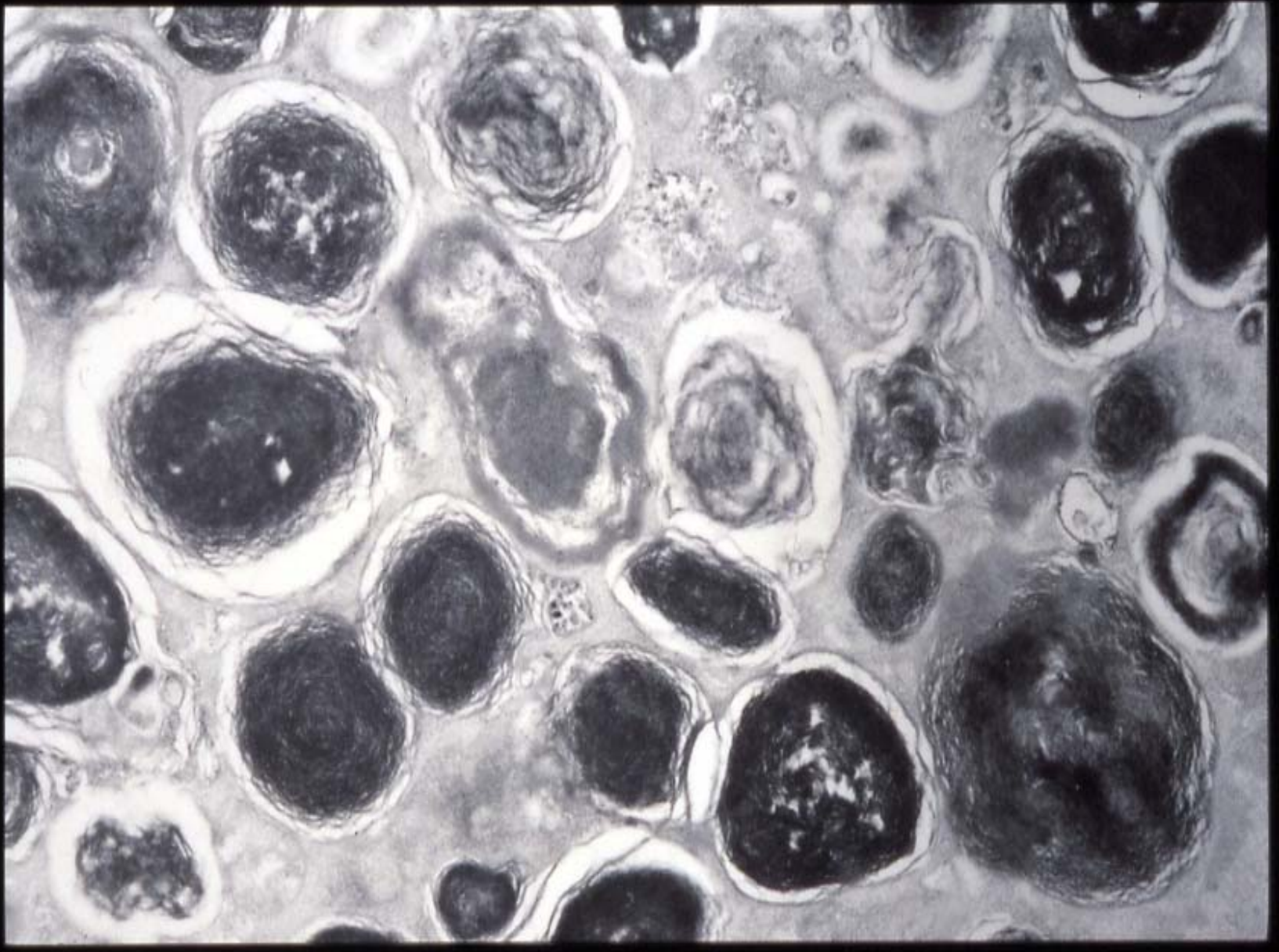
Alveolar macrophages from rats treated with chlorphentermine. Some cells are markedly enlarged and appear vacuolated (foamy), and some are multinucleated.



TEM of a control rat alveolar macrophage



TEM of a phospholipidotic alveolar macrophage from a rat treated with chlorphentermine. Note numerous electron-dense lamellar inclusions.



Lysosomal lamellar bodies in an alveolar macrophage from a rat treated with chlorphentermine

EFFECT OF AMIODARONE TREATMENT ON RAT PULMONARY PHOSPHOLIPIDS (150 mg/kg)

Treatment Group	<u>Class of Phospholipid</u> (values are % of Control)					
	PC	PE	SP	PS	PI	PG
1 Week	102	90	113	86	85	86
3 Weeks	250	160	152	218	221	143
9 Weeks	280	200	200	236	226	150
16 Weeks	343	165	163	186	190	171

PC-phosphatidylcholine; PE-phosphatidylethanolamine; SP-sphingomyelin;

PS-phosphatidylserine; PI-phosphatidylinositol; PG-phosphatidylglycerol

$P < 0.05$ vs Control (*Reasor et al., Tox. Appl. Pharmacol. 97:124-133, 1989*)

Phospholipid and Drug Content of Rat Alveolar Macrophages During Recovery from Amiodarone (AD) Administration

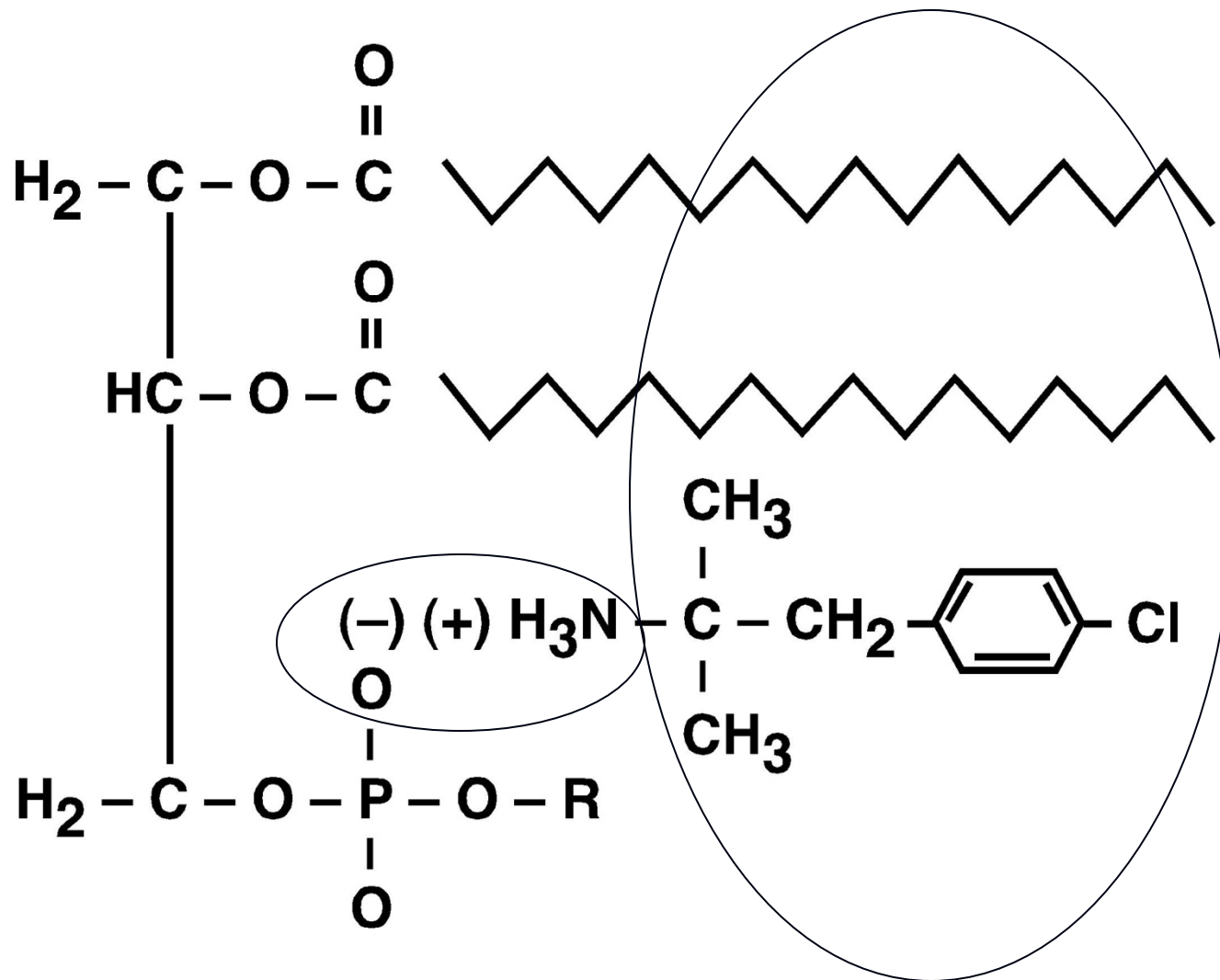
Treatment Group	Total Phospholipid ($\mu\text{moles}/10^7$ cells)	AD ($\mu\text{moles}/10^7$ cells)	DesethylAD ($\mu\text{moles}/10^7$ cells)
Control	0.56 ± 0.06	--	--
3 weeks AD	$3.17 \pm 0.37^*$	118 ± 12	170 ± 16
3 weeks AD + 1 week recovery	0.60 ± 0.05	9 ± 2	15 ± 5
3 weeks AD + 2 weeks recovery	0.45 ± 0.06	3 ± 1	8 ± 4

* $P < 0.05$ *Reasor, et al., 1988*

Possible Mechanisms by Which Lamellar Inclusions are Induced by CADs

➤ Impairment in lysosomal enzyme activity

- Drug-phospholipid interaction resulting in an indigestible or charge-neutralized substrate
- Drug-enzyme interaction resulting in direct inhibition of lysosomal phospholipase activity



**Proposed Drug – Phospholipid Interaction
 Leading to Accumulation of Phospholipid
 Within the Cell**

Possible Mechanisms by Which Lamellar Inclusions are Induced by CADs

Other processes

- Increase in phospholipid synthesis
- Impaired membrane recycling and lysosomal enzyme sorting dynamics

*The landmark genomic study by Sawada, H., Takami, K., & Asahi, S. Toxicol. Sci. 83:282-292, (2005) described 12 genes **associated** with the induction of phospholipidosis that could be classified in four functional categories that are involved in **down-regulated phospholipase activity and lysosomal enzyme transport as well as up-regulation of cholesterol and phospholipid biosynthesis.***

There may be more than one mechanism occurring for a given drug and the mechanism(s) may be different among drugs.

Toxicological/Functional Consequences

- Tissue damage has been observed in association with the induction of phospholipidosis by some drugs.
- Studies have been conducted *in vivo* and *ex vivo* in an attempt to determine if the induction of drug-induced phospholipidosis is causally related to impairment in tissue or cellular function.
- Results have been variable, some studies showing impaired function, some no change in function and some enhanced function. These changes have not been definitively linked to the presence of phospholipidosis in the cell or tissue.
- Some studies with gentamicin, a drug which does not have a typical cationic amphiphilic structure, but causes phospholipidosis in the kidney, are suggestive of toxicity, but a causal relationship has not been established.

Toxicological/Functional Consequences

- The prevailing theory is that drug-induced phospholipidosis is an adaptive response to the presence of the drug and is not a toxic condition. Except for a few studies including the recent study involving neurophospholipidosis induced by posaconazole in dogs, the studies to date have not been adequate to draw any definitive conclusions about this or any other theory.

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Phospholipidosis in Neurons Caused by Posaconazole, without Evidence for Functional Neurologic Effects

MARK E. CARTWRIGHT,¹ JANET PETRUSKA,¹ JOSEPH AREZZO,² DOYLE FRANK,¹ MONA LITWAK,² RICHARD E. MORRISSEY,¹
JAMES MACDONALD,¹ AND THOMAS E. DAVIS¹

¹*Schering-Plough Research Institute, Lafayette, NJ 07848*

²*Albert Einstein School of Medicine, Bronx, NY 10461*

ABSTRACT

The azole antifungal drug posaconazole caused phospholipidosis in neurons of the central nervous system, dorsal root ganglia of the spinal cord, and myenteric plexus in chronic toxicity studies in dogs. The time of onset, light and electron microscopic features, neurologic and electrophysiologic effects on the central and peripheral nervous systems, and potential for regression were investigated in a series of studies with a duration of up to one year. Nuclei of the medulla oblongata were the prominently affected areas of the brain. Neurons contained cytoplasmic vacuoles with concentrically whorled plasma membrane-like material (i.e., multilamellar bodies) morphologically identical to that commonly caused in other tissues by cationic amphiphilic drugs. Some axons in the brain and spinal cord were swollen and contained granular eosinophilic, electron-dense lysosomes. There were no features suggesting degeneration or necrosis of neurons or any associated elements of nervous tissue. The earliest and most consistent onset was in neurons of dorsal root ganglia. The observed neural phospholipidosis did not result in any alteration in the amplitude or latency of the auditory, visual, or somatosensory evoked potentials. The histopathologic changes did not progress or regress within the three-month postdose period. The results indicate that phospholipidosis can be induced in central and peripheral neurons of dogs by administration of posaconazole, but this change is not associated with functional effects in the systems evaluated.

Keywords: phospholipidosis; neurons; posaconazole; dog; function.

Toxicological/Functional Consequences

- When phospholipidosis is observed in cells or tissues, the need, and challenge, are to design studies that will assess the function of the affected tissue and establish whether any changes in function, if present, are causally related to the phospholipidosis.

Summary/Conclusions

- Phospholipidosis is a response that can be induced *in vivo* and *ex vivo* by a number of drugs with cationic amphiphilic structures and is a significant impediment in drug development.
- The ability to induce phospholipidosis is unrelated to the pharmacologic activity of a drug.
- The species and tissue/cellular susceptibilities are unpredictable based on the structure of a drug.
- Inhibition of lysosomal metabolism of phospholipids plays an important role in the development of phospholipidosis, but may not be the only mechanism involved.

Summary/Conclusions (con't)

- Generalities regarding the etiology, incidence, and effect of a drug on a specific host should not be made. Each drug must be evaluated separately.
- The toxicological significance of phospholipidosis is unclear. There is no definitive evidence to directly link the presence of this condition to impaired cellular or tissue function in intact organisms. Nevertheless, due to the marked changes in cellular morphology associated with phospholipidosis, efforts should be directed at designing studies that will provide relevant information on this issue.

Summary/Conclusions (con't)

- Until further research addresses the uncertainties associated with phospholipidosis, the pharmaceutical industry will continue to face questions from the U.S. F.D.A. and regulatory agencies abroad.
- In all areas of research, efforts to extrapolate data from animal studies to humans are essential.

STATEMENT FROM THE SOCIETY OF TOXICOLOGIC PATHOLOGY AND REGULATORY POLICY COMMITTEE'S PHOSPHOLIPIDOSIS INTEREST GROUP

- “We are likely to continue to discharge potentially beneficial compounds from development until we have a better mechanistic understanding of the causes and implications of phospholipid accumulation and use this understanding to develop clinically useful biomarkers and informed risk assessments.”

Berridge, B.R., et al., *Toxicologic Pathology* 35: 325 (2007)

Bis(monoacylglycerol)phosphate as a Biomarker to Evaluate Phospholipidosis for Drug Risk Management

FDA Pharmaceutical Science & Clinical Pharmacology Advisory Committee
April 14, 2010

Frank Hsieh, Ph.D.
frank.hsieh@nextcea.com



600 West Cummings Park, # 6375, Woburn, MA 01081
www.nextcea.com

Presentation Outline

1. Why are PL biomarkers needed?
2. Identified In-vivo BMP biomarker to evaluate PL in drug development
3. PL biomarker (BMP species) validation in humans

Why are PL Biomarkers needed?

Technology Points of View:

- To date, the determination of PL relies on transmission electron microscopy (EM) examination of tissues.
- It is difficult to monitor the time course of PL in individual animals or humans with EM.
- Tissue biopsy for routine investigation of PL may not be feasible in the clinic.

Drug Safety Points of Views:

- Need to demonstrate whether PL is an adaptive response or early event in drug toxicity (ex. Is there a relationship between gentamicin induced PL and nephrotoxicity?)
- The significance of PL findings in animal studies is unknown for humans (for examples, as indicated in drug labeling).

Drug Labeling

KETEK[®] (Telithromycin, is a semi-synthetic antibacterial agent used for treatment of community-acquired pneumonia).

Sanofi-Aventis

ANIMAL PHARMACOLOGY

Phospholipidosis (intracellular phospholipid accumulation) affecting a number of organs and tissues (e.g., liver, kidney, lung, thymus, spleen, gall bladder, mesenteric lymph nodes, GI-tract) has been observed with the administration of telithromycin in rats at repeated doses of 900 mg/m²/day

..... **The significance of these findings for humans is unknown.**

PROZAC[®] (Fluoxetine Hydrochloride, is a psychotropic agent used for treatment of depression and pre menstrual dysphoric disorder, Sarafem[®]).

Eli Lilly and Company

ANIMAL TOXICOLOGY

Phospholipids are increased in some tissues of mice, rats, and dogs given fluoxetine chronically. This effect is reversible after cessation of fluoxetine treatment. Phospholipid accumulation in animals has been observed with many cationic amphiphilic drugs, including fenfluramine, imipramine, and ranitidine.

..... **The significance of this effect in humans is unknown.**

ZITHROMAX[®] (Azithromycin, is a macrolide antibiotic agent used for treatment of bacterial infection)

Pfizer

ANIMAL TOXICOLOGY

Phospholipidosis (intracellular phospholipid accumulation) has been observed in some tissues of mice, rats, and dogs given multiple doses of azithromycin. It has been demonstrated in numerous organ systems (e.g., eye, dorsal root ganglia, liver, gallbladder, kidney, spleen, and pancreas) in dogs treated with azithromycin.....

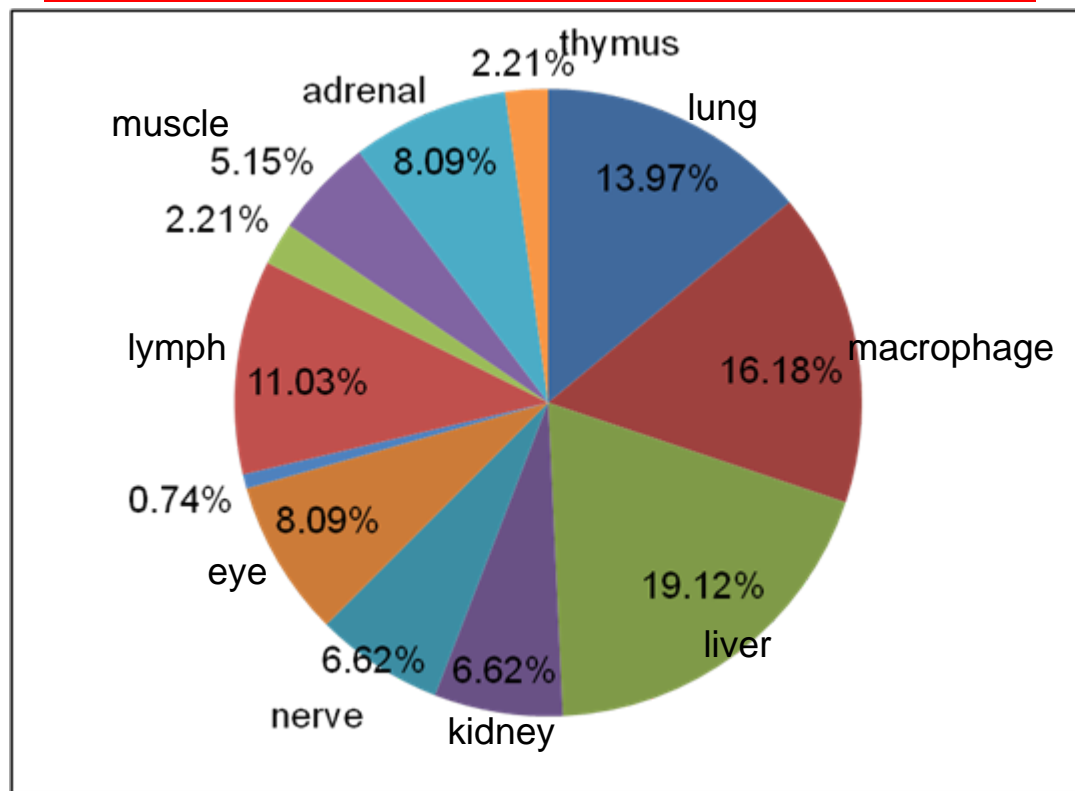
..... **The significance of these findings for animals and for humans is unknown.**

In addition, the PL rate of reversal in animals (and in humans)?

Examples of PL drugs in animals

1-chloro-10,11-dehydroamitriptyline	Hydroxyzine
1-chloroamitriptyline	Imipramine
20,25 diazacholesterol	Indoramin
6-hydroxydopamine	Iprindole
Amikacin	Ketoconazole
Amitriptyline	Labetalol
Boxidine	Telithromycin (KETEK)
Chlorcyclizine	Maprotiline
Chlorphentermine	Meclizine
Chlorpromazine	Netilmicin
Citalopram	Norchlorcyclizine
Clindamycin	Paraquat
Clomipramine	Phenacetin
Clozapine	Quinacrine
Cyclizine	Suramin
Dibekacin	Tamoxifen
Di-isobutamide	Triparanol
Emetine	Trospectomycin sulfate
Erythromycin	
Ethyl fluclozepate	Verapamil
Fenfluramine	Zimelidine
Fluoxetine (POZAC)	Azithromycin (ZITHROMAX)
Homochlorcyclizine	

Percentage of example drugs that cause PL in different animal tissues.



Similar PL profiles in human tissues?

Drugs Induced Phospholipidosis in Humans

(reported in literature, but not in drug labeling)

Drugs	Therapeutic Class
Amiodarone (Cordarone)	Antiarrhythmic drugs
Amodiaquine (Camoquin, Flavoquine)	Anti-malarial and Anti-inflammatory agent
Chloroquine (Aralen)	Anti-malarial, RA, and Lupus
4,4'-Diethylaminoethoxyhexestrol Dihydrochloride (Coralgil)	Coronary dilator
Gentamicin (Garamycin)	Aminoglycoside antibiotic
2-(2,2-dicyclohexylethyl)piperidine (Perhexiline)	Prophylactic antianginal agent
4:4'-Diamidino Stilbene (Stilbamidine)	Anti-protozoal agent
Tilorone (Amixin IC)	Antiviral
Tobramycin (TOBI)	Aminoglycoside antibiotic
Desipramine (Norpramin)	Anti-depressant
Pentamidine (Nebupent)	Anti-microbial

Questions for these PL drugs in humans:

Data on time-course and rate of reversal of PL in humans ?

Long term effects of PL on tissue function (drugs for chronic diseases) ?

Drug Labeling about Phospholipidosis

Wyeth/Pfizer

Cordarone® Intravenous (Amiodarone Hydrochloride): None

Cordarone® Oral (Amiodarone Hydrochloride)

Phospholipidosis (foamy cells, foamy macrophages), due to inhibition of phospholipase, will be present in most cases of amiodarone induced pulmonary toxicity; however, these changes also are present in approximately 50% of all patients on amiodarone therapy. These cells should be used as markers of therapy, but not as evidence of toxicity.....



Inconsistent

Published Phospholipidosis Papers

Examples:

“Progressive renal insufficiency associated with amiodarone-induced phospholipidosis”, *Kidney International*, 2008, 74, 1354-1357.

Marchlinski, F. E.; Gansler, T. S.; Waxman, H. L.; Josephson, M. E. Amiodarone Pulmonary Toxicity. *Ann. Intern. Med.* 1982, 97, 839-45.

Questions: Known PL drugs in animals, but UNKNOWN in humans

PL Drugs in Animals

1-chloro-10,11-dehydroamitriptyline	Hydroxyzine
1-chloroamitriptyline	Imipramine
20,25 diazacholesterol	Indoramin
6-hydroxydopamine	Iprindole
Amikacin	Ketoconazole
Amitriptyline	Labetalol
Boxidine	Telithromycin (Ketek)
Chlorcyclizine	Maprotiline
Chlorphentermine	Meclizine
Chlorpromazine	Netilmicin
Citalopram	Norchlorcyclizine
Clindamycin	Paraquat
Clomipramine	Phenacetin
Clozapine	Quinacrine
Cyclizine	Suramin
Dibekacin	Tamoxifen
Di-isobutamide	Triparanol
Emetine	Trospectomycin sulfate
Erythromycin	
Ethyl fluclozepam	Verapamil
Fenfluramine	Zimelidine
Fluoxetine (PROZAC)	Azithromycin (ZITHROMAX)
Homochlorcyclizine	

Preclinical Drug Candidates?

PL occurred in Humans?
PL recovery rate in Humans?
PL chronic effects on tissues?

?



PL Drugs in Humans

Amiodarone (Cordarone)
Amodiaquine (Camoquin, Flavoquine)
Chloroquine (Aralen)
4,4'-Diethylaminoethoxyhexestrol Dihydrochloride (Coralgil)
Gentamicin (Garamycin)
2-(2,2-dicyclohexylethyl)piperidine (Perhexiline)
4:4'-Diamidino Stilbene (Stilbamidine)
Tilorone (Amixin IC)
Tobramycin (TOBI)
Desipramine (Norpramin)
Pentamidine (Nebupent)

IND/NDA Drug Candidates?

Drug-Drug Interactions on PL:

Guidance for Industry

Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling

Table 2. Examples of in vivo substrate, inhibitor, and inducer for specific CYP enzymes recommended for study (oral administration) ^(1,2)

CYP	Substrate	Inhibitor	Inducer
1A2	theophylline, caffeine	fluvoxamine	smokers versus non-smokers ⁽³⁾
2B6	efavirenz		rifampin
2C8	repaglinide, rosiglitazone	gemfibrozil	rifampin
2C9	warfarin, tolbutamide	fluconazole, amiodarone (use of PM versus EM subjects) ⁽⁴⁾	rifampin
2C19	omeprazole, esoprazole, lansoprazole, pantoprazole	omeprazole, fluvoxamine, moclobemide (use of PM versus EM subjects) ⁽⁴⁾	rifampin
2D6	desipramine, dextromethorphan, atomoxetine	paroxetine, quinidine, fluoxetine (use of PM versus EM subjects) ⁽⁴⁾	none identified
2E1	chlorzoxazone	disulfiram	ethanol
3A4, 3A5	midazolam, buspirone, felodipine, lovastatin, eletriptan, sildenafil, simvastatin, triazolam	atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin	rifampin, carbamazepine

Biomarkers to Evaluate Phospholipidosis for Drug Safety Management

Biomarkers to Monitor Drug-Induced Phospholipidosis

(Toxicology and Applied Pharmacology, 218:72-78.
2007)

Objective: Identify biomarkers associated with the occurrence of tissue PL in rats treated with 10-day repeat dose of amiodarone, azithromycin or gentamicin

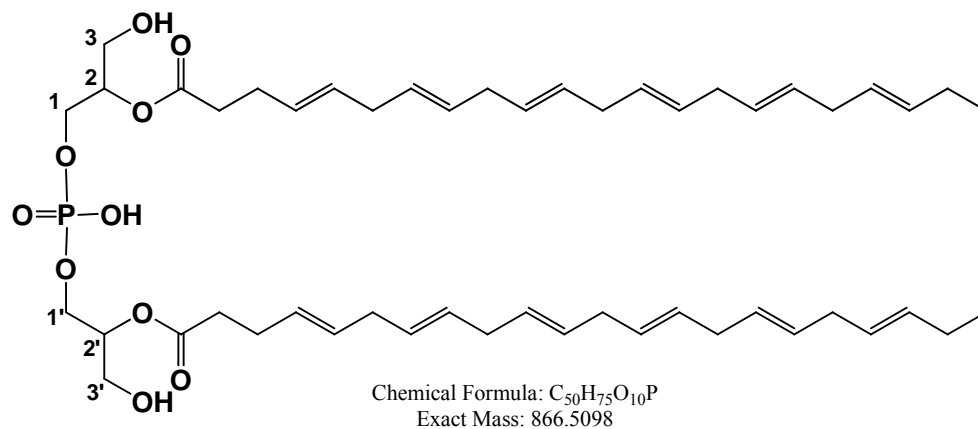
Study Design: Rats received either amiodarone (150 mg/kg), azithromycin (30 mg/kg) or gentamicin (100 mg/kg) once daily, IV dose, for 10 consecutive days; Occurrence of tissue PL in drug treated rats (but not controls) shown by EM

Histopathology (EM):

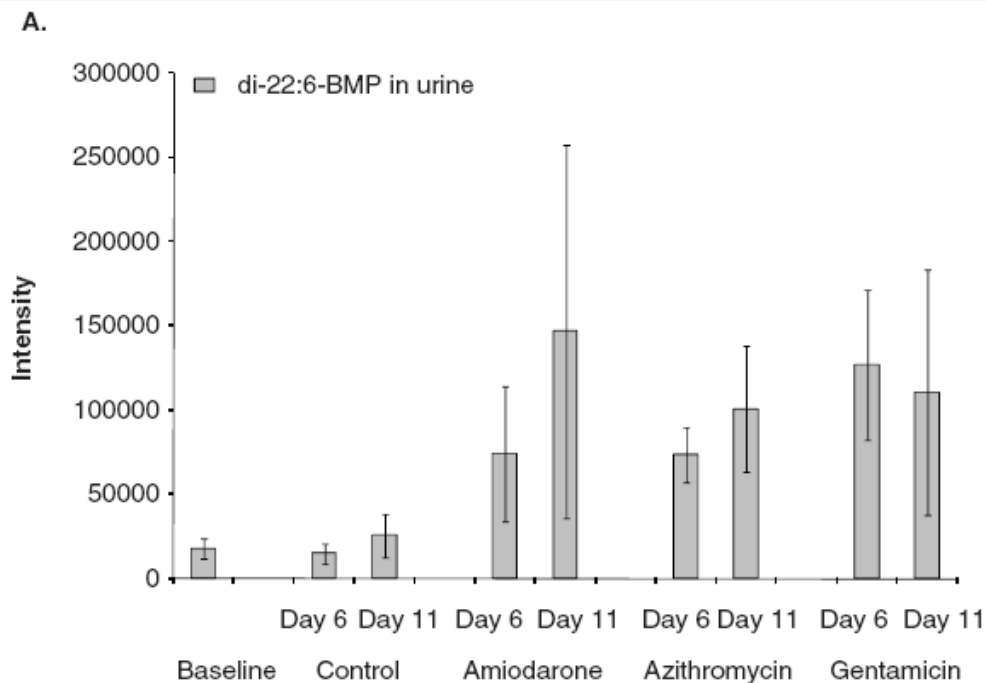
Histological examination of rat tissues by electron microscopy (Day 11). Relative accumulation of lamellar inclusions: – not observed, + slight (very few), ++ mild (some), +++ moderate (several)

Tissue	Control	Amiodarone	Azithromycin	Gentamicin
Liver	-	+	-	+++
Lung	-	+++	++	++
Kidney	-	+++	++	+++
Lymph Node	-	+	-	+

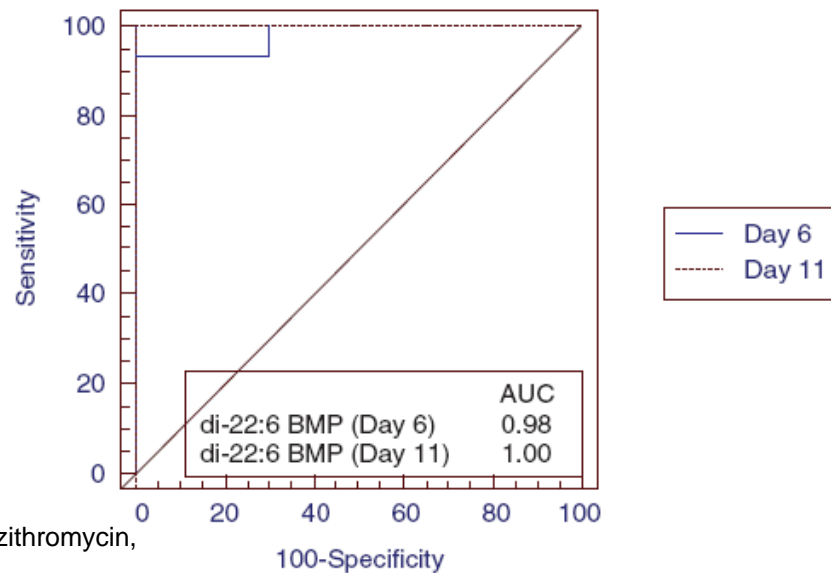
In-vivo Drug-Induced Phospholipidosis Biomarker



Di-22:6 Bis(monoacylglycerol)phosphate



B. All histopathology phospholipidosis grades

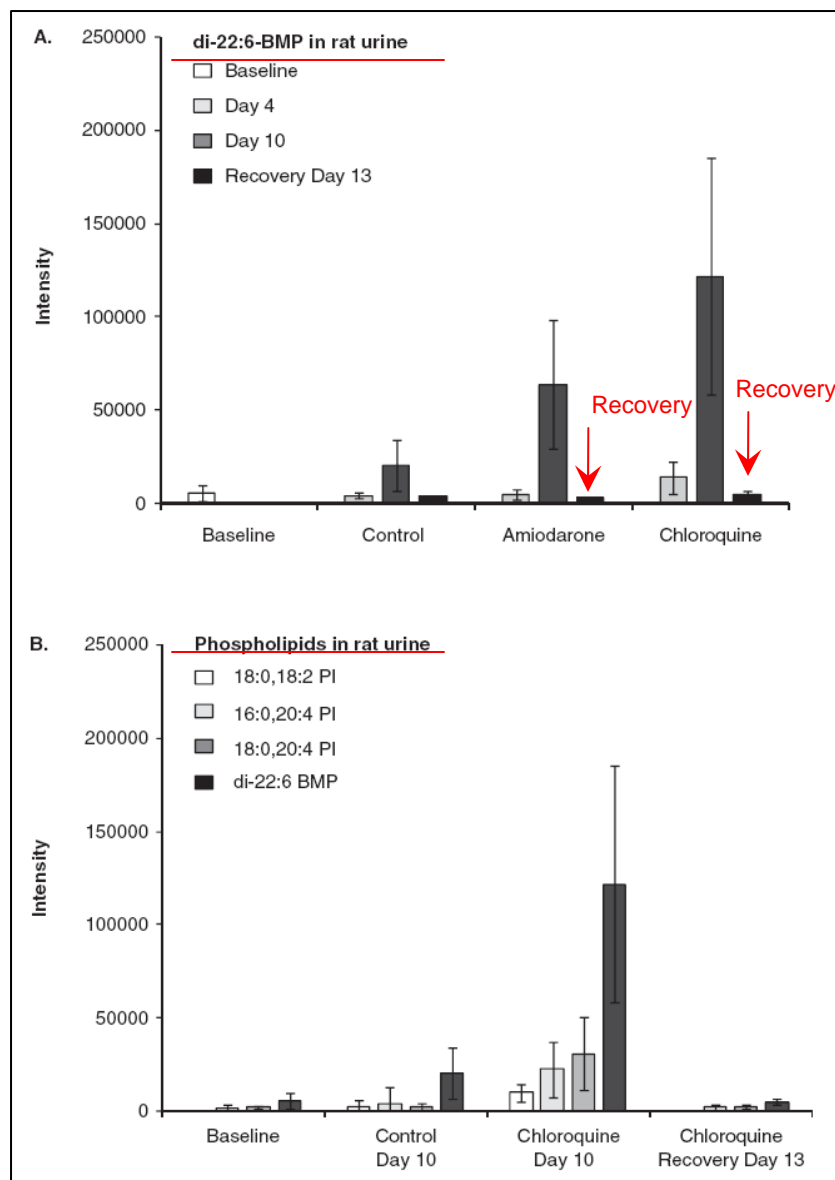


(Amiodarone, Azithromycin,
& Gentamicin)

(Expert Opinion in Drug
Metabolism and Toxicology, 2010,
Tengstrand, Miwa, and Hsieh,
April, 2010, p. 1-16)

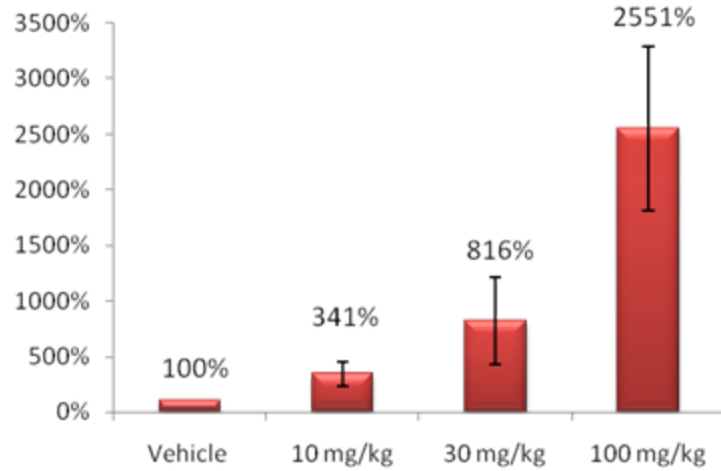
Bis(monoacylglycerol)phosphate as a non-invasive biomarker in urine to monitor the onset and time-course of phospholipidosis with drug-induced toxicities

(Expert Opinion in Drug Metabolism and Toxicology, 2010, Tengstrand, Miwa, and Hsieh, April, 2010, p.1-16)

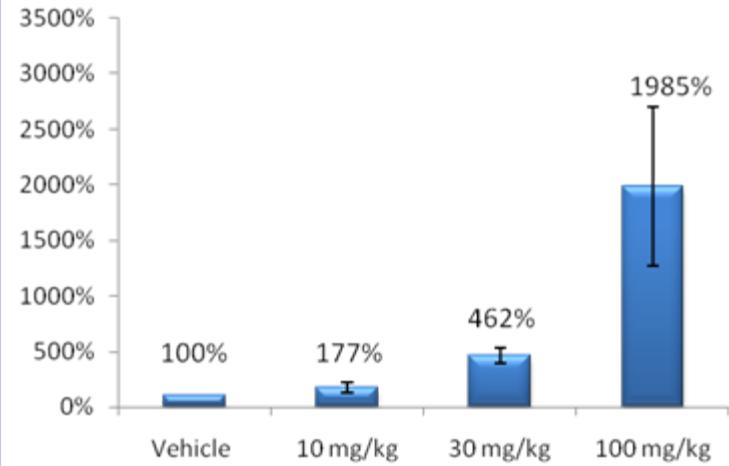


PL Biomarker, Di-22:6-BMP, in Rat Tissues

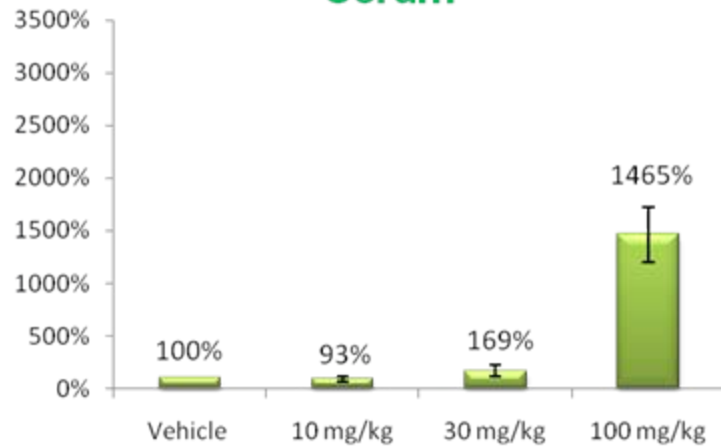
Lung



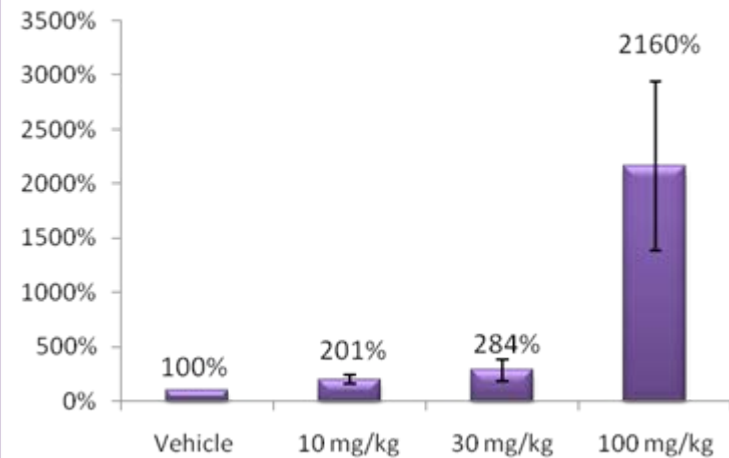
Liver



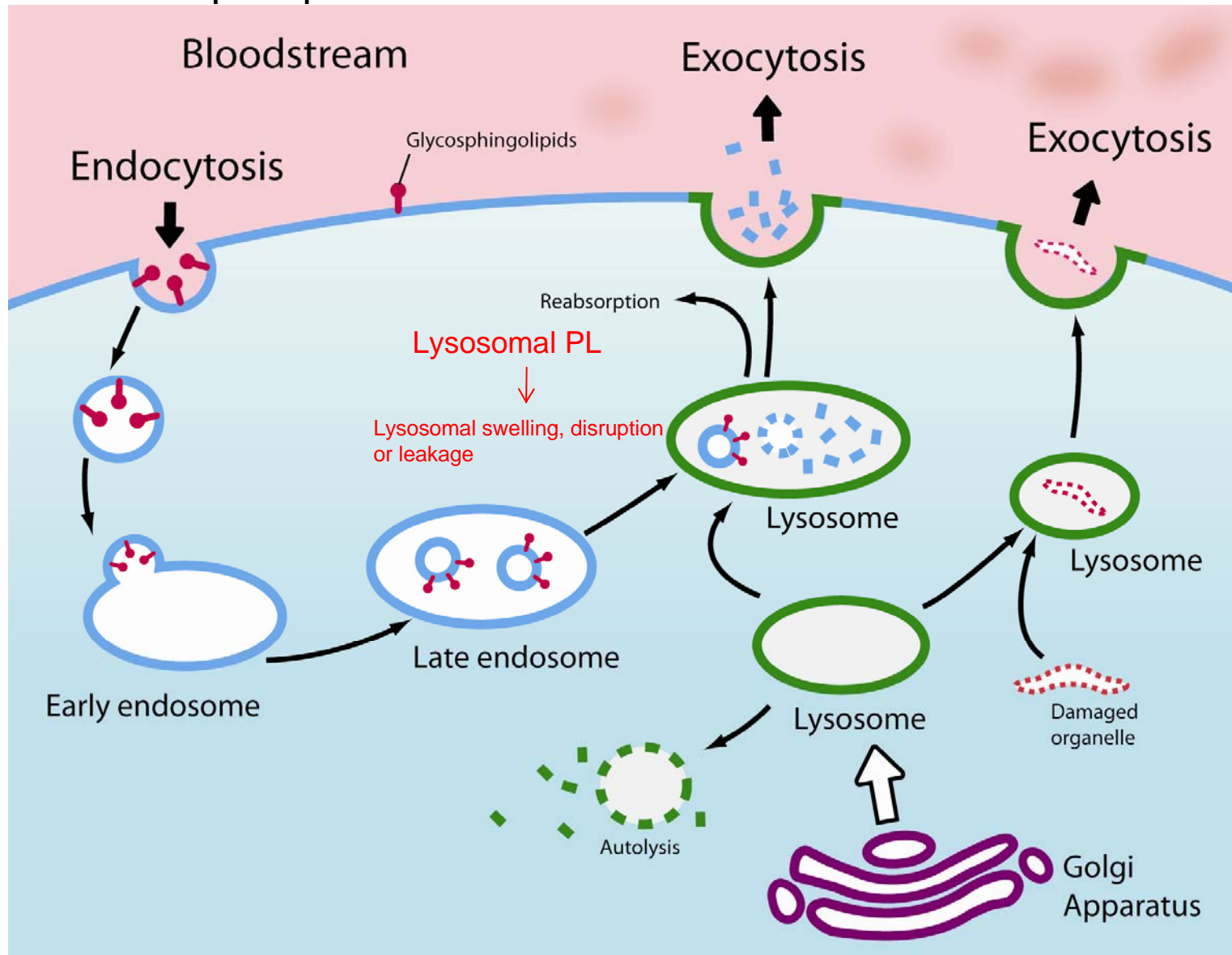
Serum



Urine

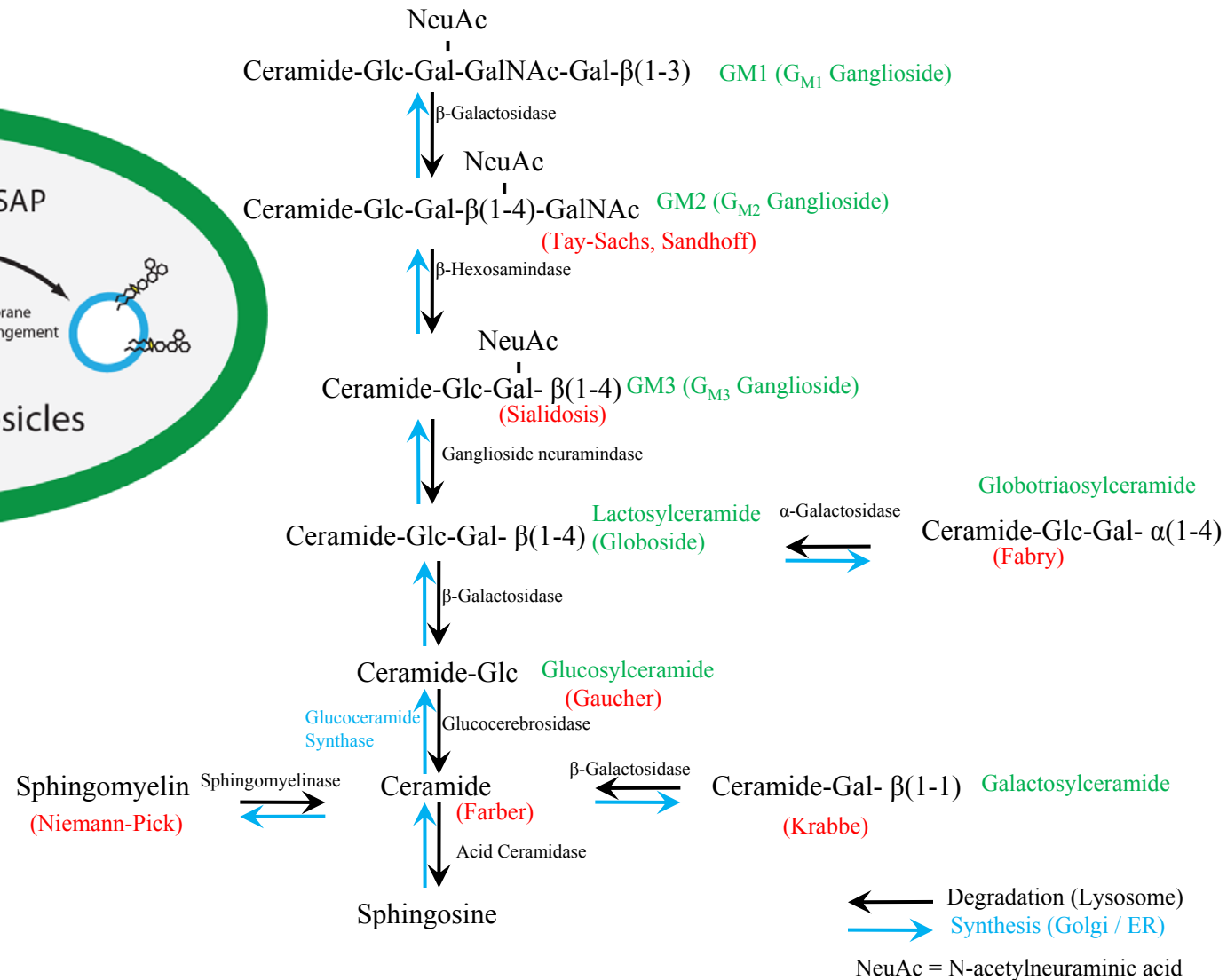
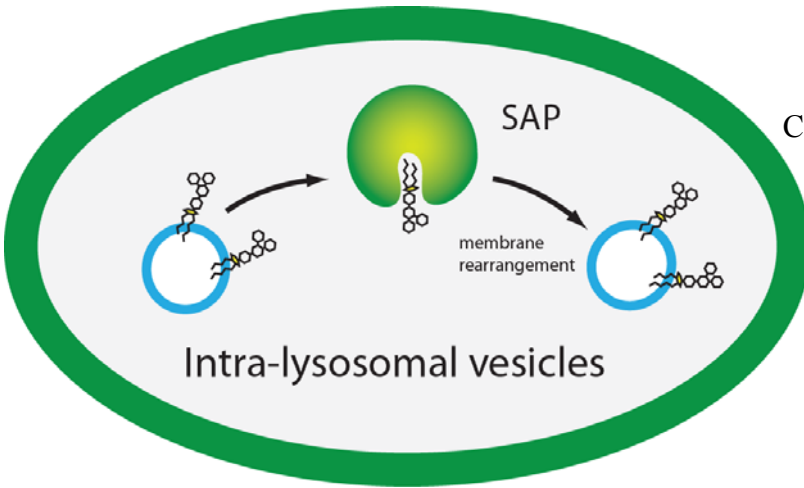


Lysosomal Phospholipidosis



BMP, anionic phospholipid, is localized on the surface of intra-lysosomal vesicles

Lysosome

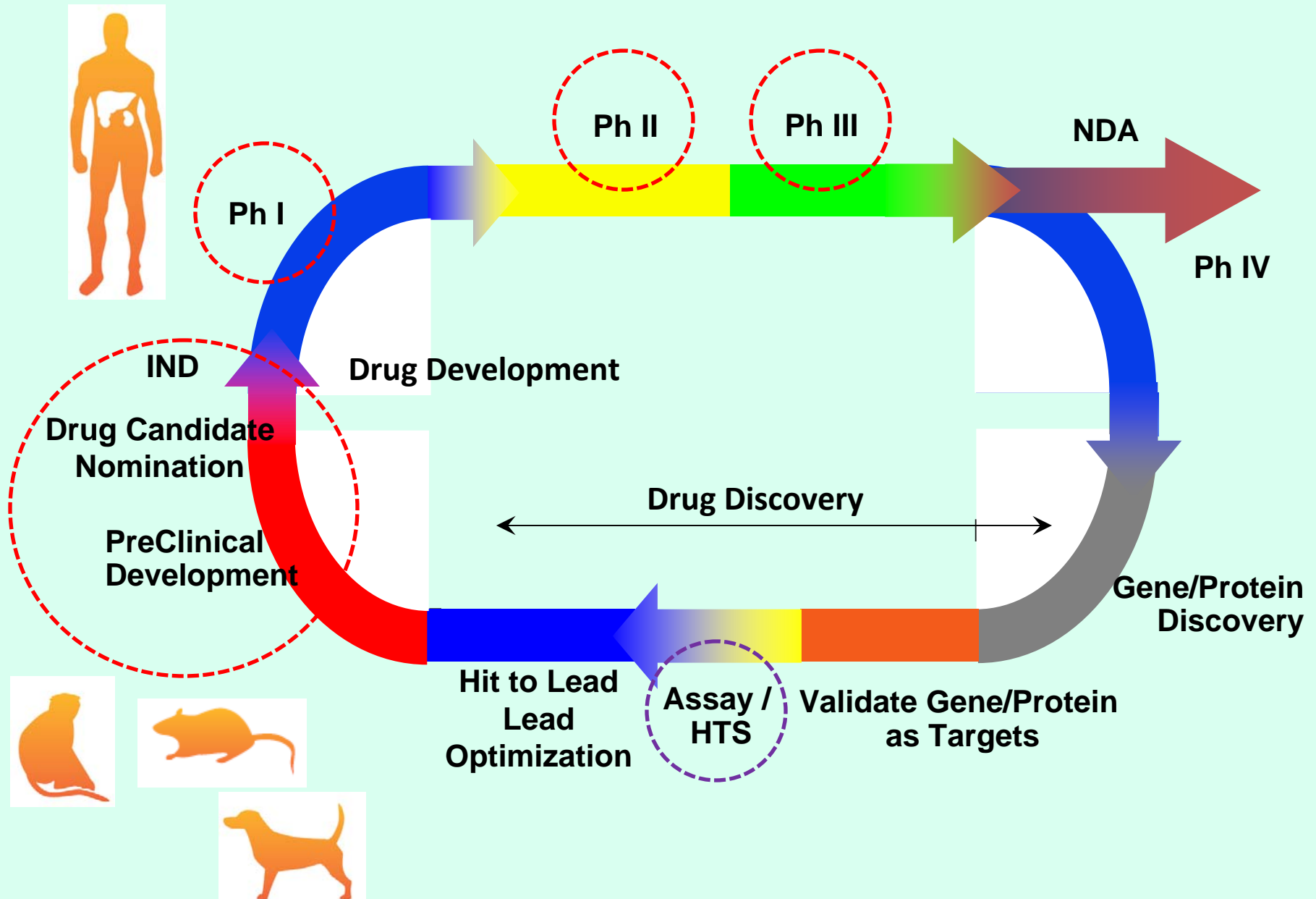


NPC Patients:

Disorder of intracellular glycosphingolipids and cholesterol trafficking resulting in endosomal/lysosomal accumulation and predominant manifestations of progressive neurodegeneration.

- Late endosomal membranes rich in BMP regulate cholesterol transport in NPC (Nature Cell Biol, 1999, 1: 113-118).
- BMP helps NPC1/NPC2 proteins transfer cholesterol (PNAS, 2008, 105(40): 15287-15292).
- BMP is an essential cofactor for lysosomal sphingomyelin metabolism (Nature, 2010, 463(28): 549-554.)

PL Biomarkers (BMP) to monitor drug-induced phospholipidosis



Validation of Phospholipidosis BMP Biomarker in Humans

Bis(monoacylglycerol)phosphate (BMP) is accumulated in the tissues of patients with drug-induced phospholipidosis and Niemann-Pick disease, type C (NPC).

Nature Cell Biology (1999) 1/2: 113-118.

Prostaglandins, Leukotrienes and Essential Fatty Acids (2009) 81: 313-324

Expert Opinion in Drug Metabolism and Toxicology (April, 2010, p. 1-16), Tengstrand, Miwa, and Hsieh,

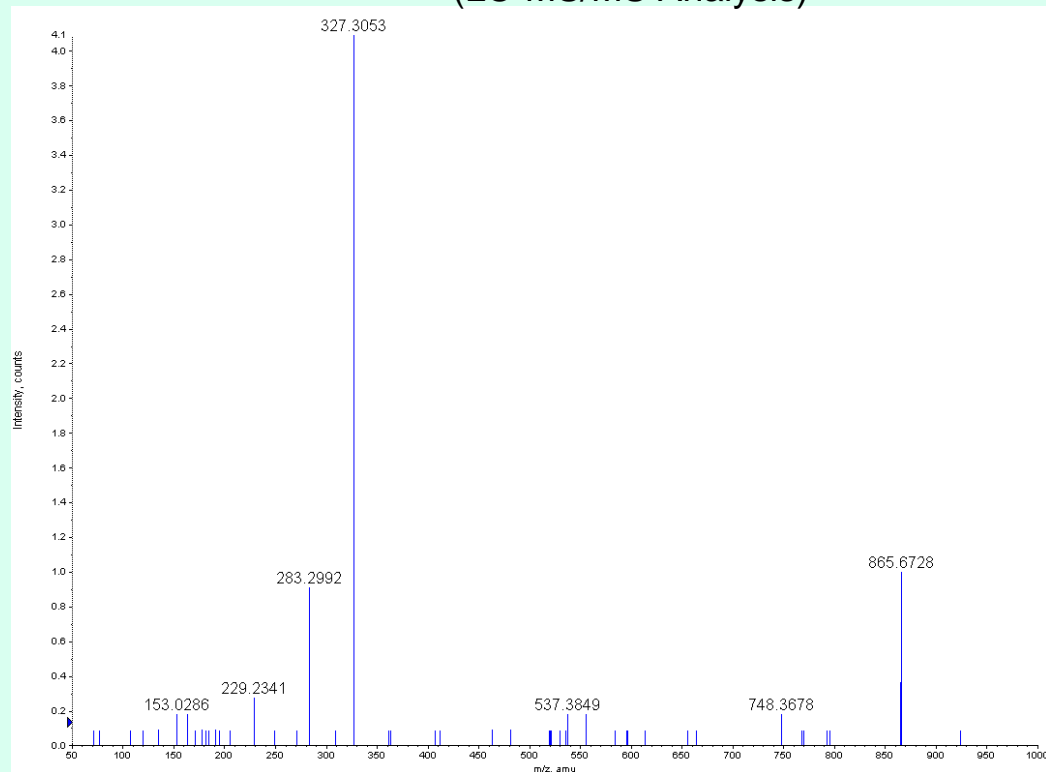
Biomarker Validation in Human (unique BMP species):

Urine samples of NPC patients with different stages of disease severity will be analyzed and validated by LC-MS/MS at Nextcea.

Is the biomarker (di-22:6 BMP) to monitor drug-induced phospholipidosis in animals also detected by LC-MS/MS in humans?

YES

Low levels of di-22:6 BMP was identified in healthy human subjects
(LC-MS/MS Analysis)



Phospholipidosis database is being developed at FDA (will contain FDA in-house preclinical and clinical data from IND / NDAs of compounds that induce phospholipidosis)

DruSafe Phospholipidosis subteam members submit data on compounds that cause phospholipidosis that either were discontinued or are currently under development

FDA
Phospholipidosis
Working Group
Initiatives



BMP Biomarker, especially, for
PL-associated Toxicity

Phospholipidosis database components include Chemistry, ADME, Toxicology, and Clinical

Biomarker to Evaluate Phospholipidosis for Drug Safety Management

PL Drugs in Animals

1-chloro-10,11-dehydroamitriptyline	Hydroxyzine
1-chloroamitriptyline	Imipramine
20,25 diazacholesterol	Indoramin
6-hydroxydopamine	Iprindole
Amikacin	Ketoconazole
Amitriptyline	Labetalol
Boxidine	Telithromycin (KETEK)
Chlorcyclizine	Maprotiline
Chlorphentermine	Meclizine
Chlorpromazine	Netilmicin
Citalopram	Norchlorcyclizine
Clindamycin	Paraquat
Clomipramine	Phenacetin
Clozapine	Quinacrine
Cyclizine	Suramin
Dibekacin	Tamoxifen
Di-isobutamide	Triparanol
Emetine	Trospectomycin sulfate
Erythromycin	
Ethyl fluclozepam	Verapamil
Fenfluramine	Zimelidine
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PL recovery rate in Humans?
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2-(2,2-dicyclohexylethyl)piperidine (Perhexiline)
4:4'-Diamidino Stilbene (Stilbamidine)
Tilorone (Amixin IC)
Tobramycin (TOBI)
Desipramine (Norpramin)
Pentamidine (Nebupent)

IND/NDA Drug Candidates?

Summary:

The di-22:6 BMP (di-docosahexaenoyl-Bis-monoacylglycerol-phosphate) may be used:

- To monitor the onset and time course of PL and to better understand the functional consequences which could contribute to the toxicities of drugs (such as QT prolongation, Myopathy, Renal toxicity)
- To evaluate the reversibility of PL in each tissue/species. At least one repeat multidose animal/human safety study (4 weeks, minimum) prior to IND/NDA filing.

FDA PLWG Activities on Phospholipidosis: Data Mining, Modeling and Laboratory Research

Presented by James M. Willard, Ph.D.
Phospholipidosis Working Group and
Division of Cardiovascular and Renal
Products - CDER

Outline

- Database Construction
- Preliminary Information from the Database
- Link of non-clinical Phospholipidosis to clinical QT prolongation
- QSAR (Quantitative Structure Activity Relationship) Modeling
- Lab Research
- BMP results

Database Creation

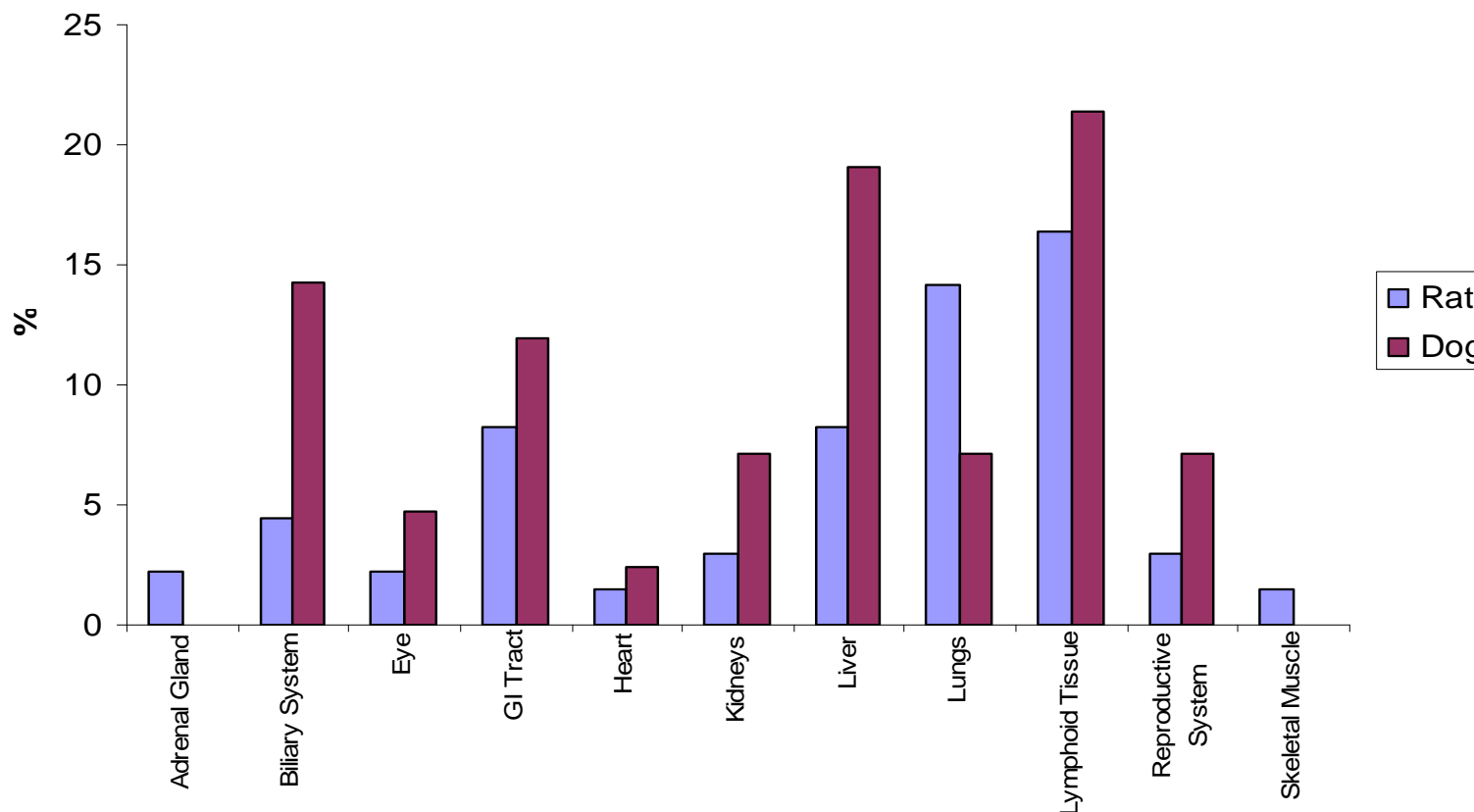
- FDA has a large repository of data from INDs and NDAs from many sponsors
- Consists of 5 tables
 - Summary - 27 Fields
 - Chemistry info (chemical structures, physical chemical data) – 11 Fields
 - ADME data (Absorption Distribution Metabolism Excretion) – 17 Fields
 - Toxicology (non-clinical) – 29 Fields
 - Clinical – 29 Fields
 - 113 Fields total

Progress.....

- 385 total drugs that induce PL
- 173 Electron Microscope confirmed PLD positive drugs
- 212 Non-EM Confirmed PLD positive drugs
- 143 are associated with New Drug Applications (NDAs - a drug may be associated with more than one NDA)
- Toxicology Table has 210 studies representing 52 compounds

Comparison of Dog (42 studies) and Rat (134 studies) Tissue Distributions in Drug-induced Phospholipidosis (% PLD positive tissues)

Rat and Dog PLD Target Organs

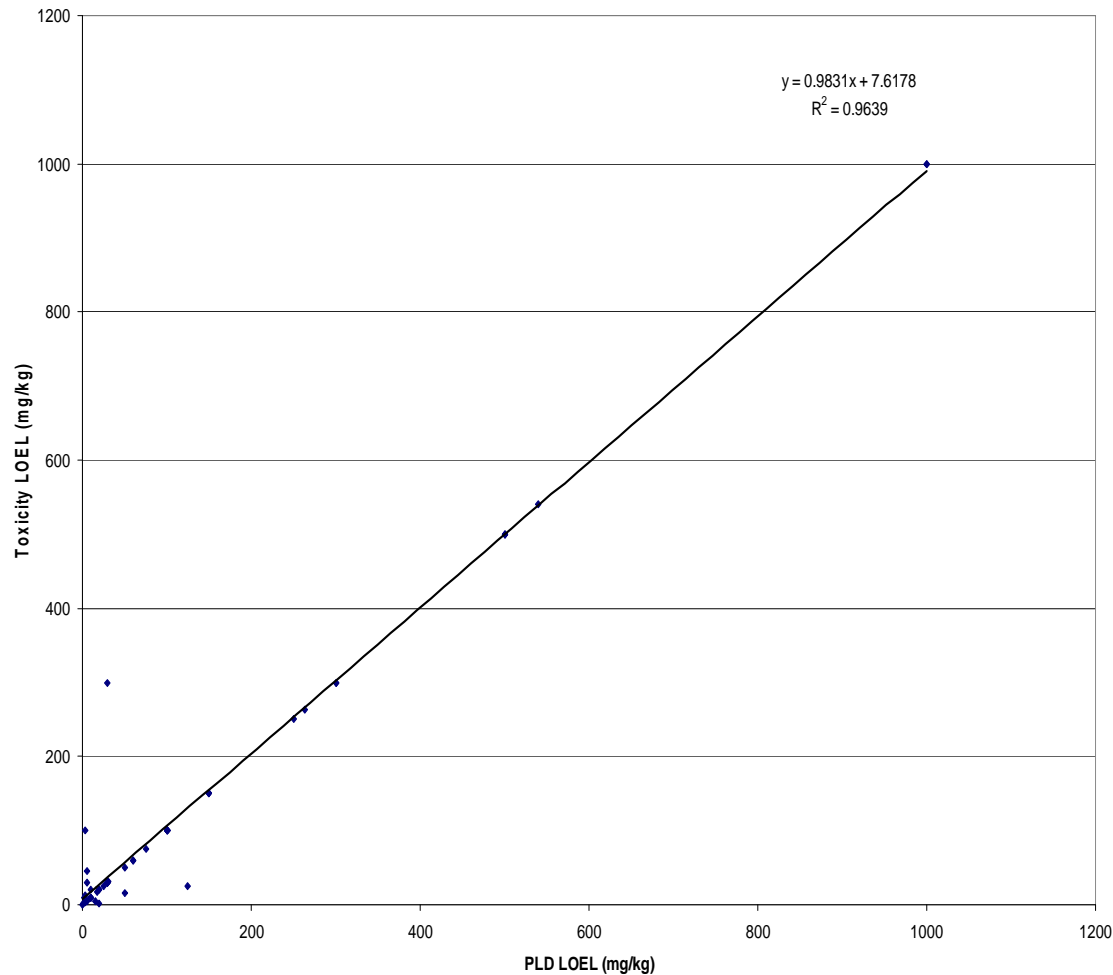


Study Species:

- 134 Rats
- 42 Dogs
- 16 Mice
- 6 Monkeys
- 3 Pigs
- 3 Rabbits
- 1 Hamster
- 1 Guinea Pig

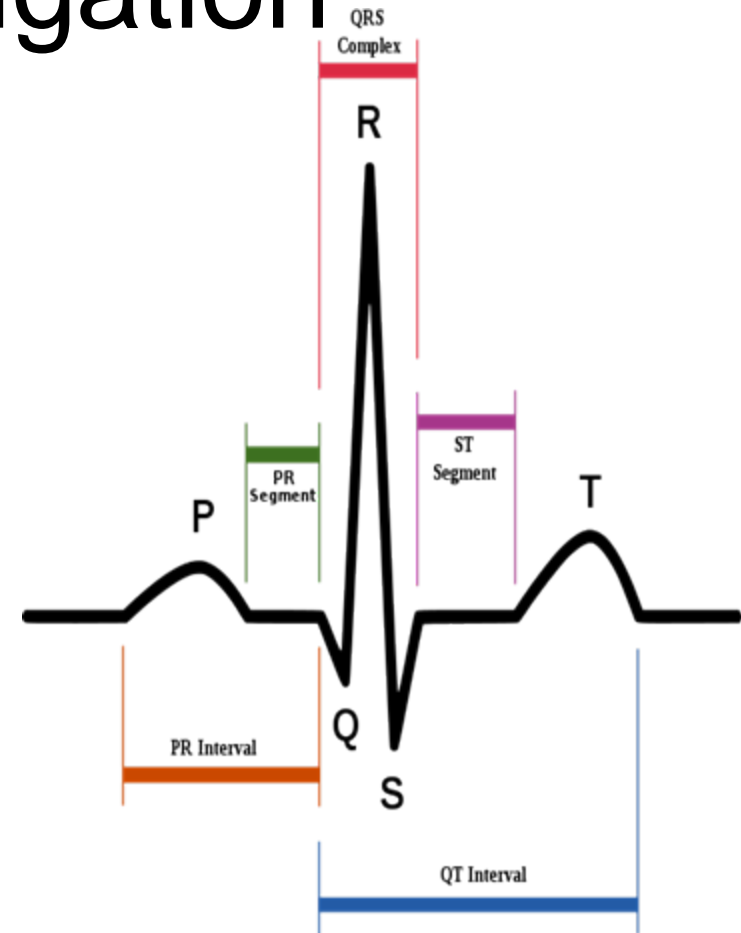
Relationship of PLD to Toxicity

PLD Presence vs. Toxicity



QT prolongation

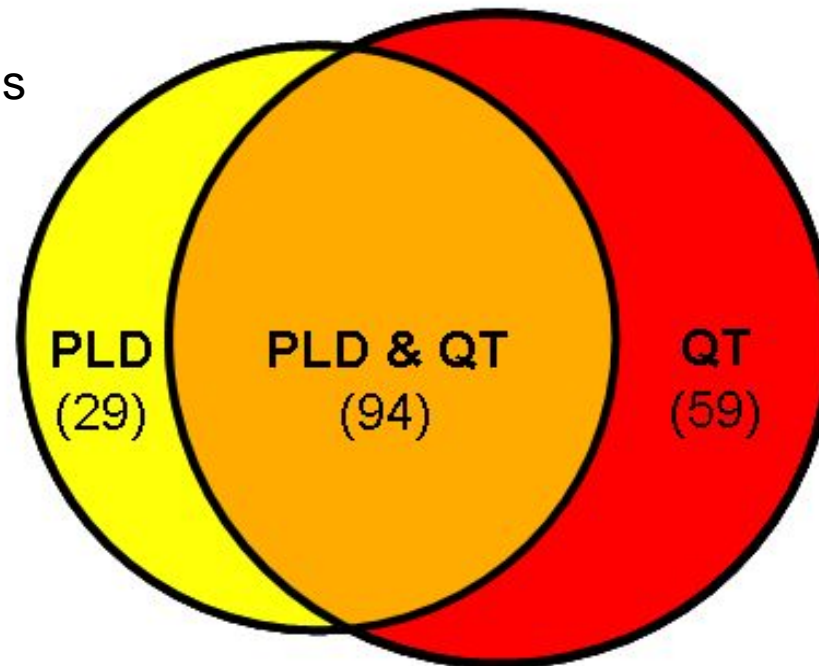
- QT prolongation indicates the part of an ECG waveform where K^+ is repolarizing the cardiac myocytes, preparing them for the next contraction
- QT prolongation is recognized as a serious issue in drug safety, primarily due to its linkage to sudden cardiac death and Torsades de Pointes





Union of FDA Phospholipidosis and CERT QT Prolongation databases

FDA Phospholipidosis
Database of
102 PLD Positive
Approved Drugs

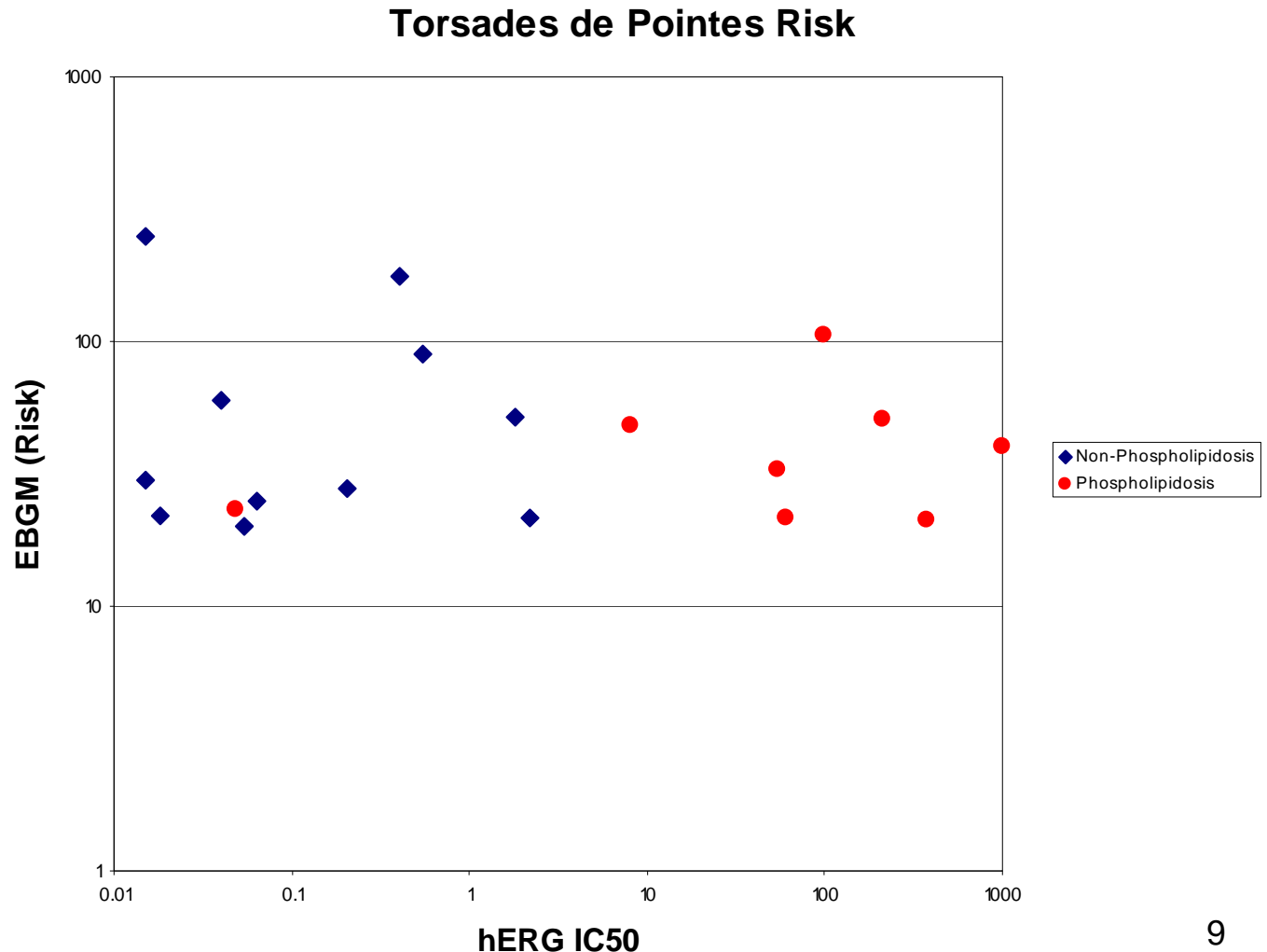


Arizona CERT
Database of
104 Drugs
With Risk of
Torsades de
Pointes

QT only drugs have primarily on-target toxicity – cardiac, PLD only drugs have little Toxicity, PLD & QT drugs have significant off-target toxicities – cardiac, hepatic.⁸

Poor hERG Blockers associated with PLD cause a disproportionate level of Torsades, While Strong hERG Blockers are the Model for Causing Torsades

Ibutilide
 Azimilide
 Leveacetylmethadole
 Bepridil
 Sotalol
 Halofantrine
 Aclarubicin
 Disopyramide
 Methadone
 Isoproterenol
 Quinidine
 Garenoxacin
 Pentamidine
 Erythromycin
 Cisapride
 Terfenadine
 Droperidol
 Astemizole
 Pimozide
 Acetyldigoxin
 Clofazimine
 Fleicanide
 Sparfloxacin
 Procainamide
 Dofetilide

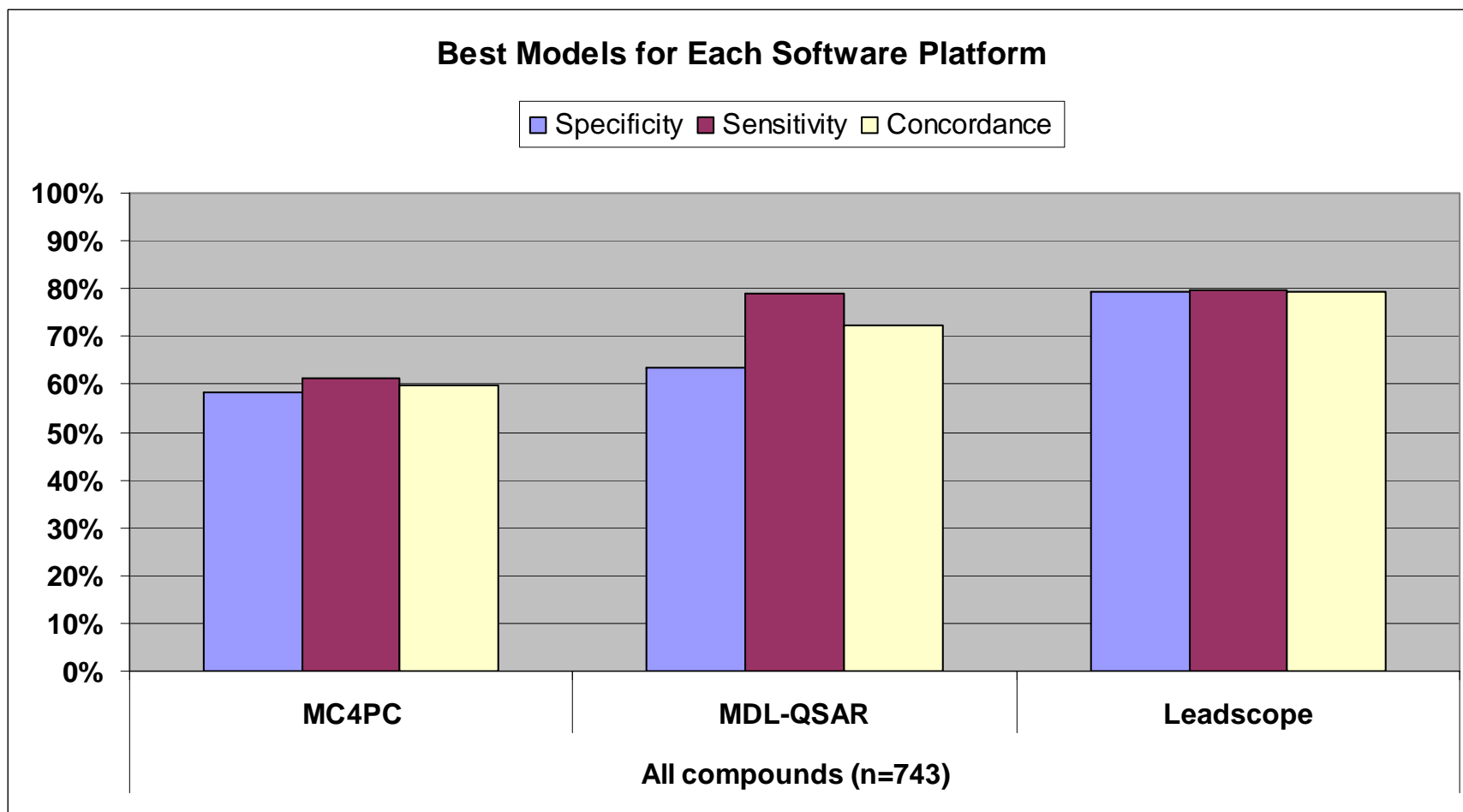




PLD Models

- (Q)SAR – Quantitative Structure Activity Relationship
 - Correlation between structural features of a molecule and activity at a given endpoint, in this case PLD
 - Requires a balanced training set of positive and negative findings
- PL models were constructed by CDER using three QSAR software programs: MC4PC, MDL-QSAR, and Leadscope
- A fourth SAR prediction software, Derek for Windows, contains a PLD structural alert that was tested for predictive performance with the PLD database

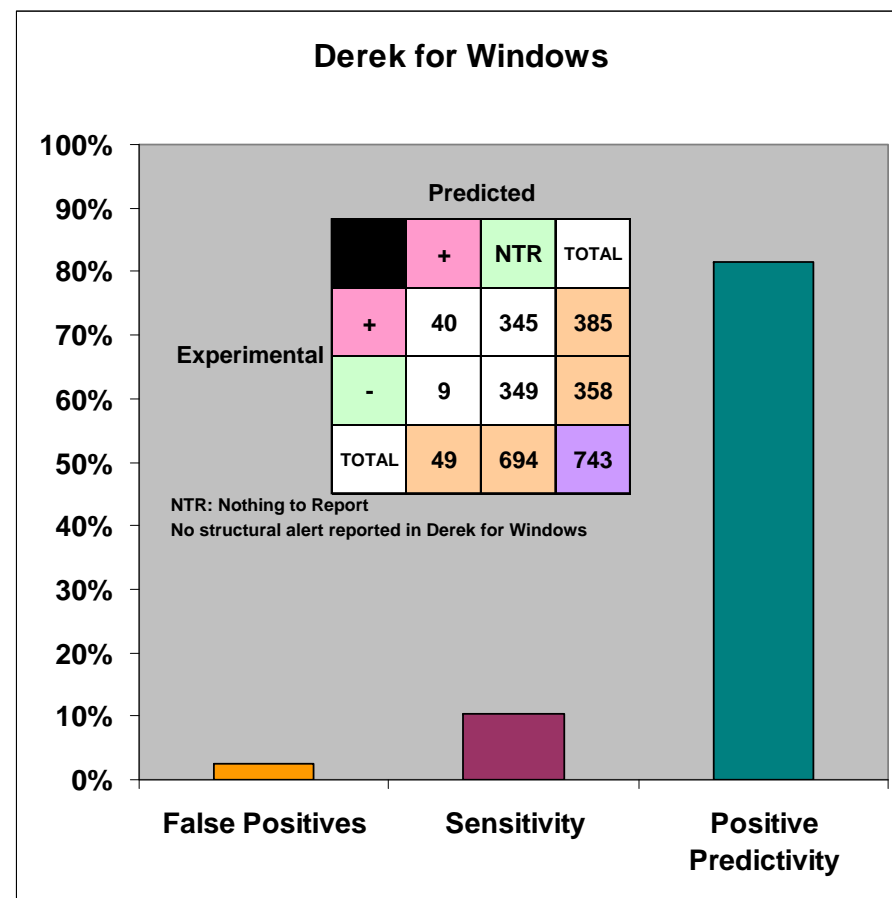
New PLD Model Results



Each Model has specific strengths and weaknesses

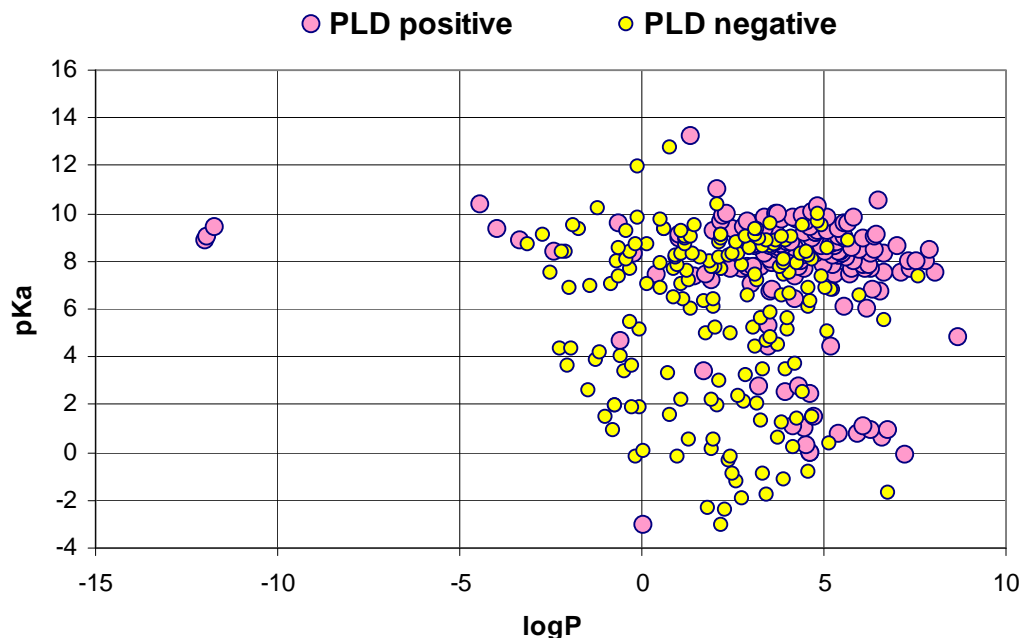
Testing SAR Model

- Derek for Windows has one structural alert for PLD based on CAD
- The alert predicts well, but could be complemented by a second alert to increase overall sensitivity

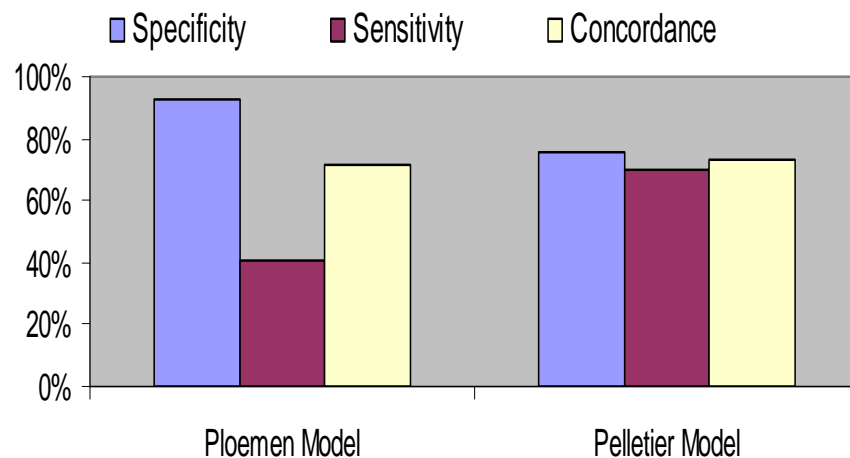


Physicochemical properties associated with PLD: pKa, logP

Relationship between pKa and logP compounds in the PLD database



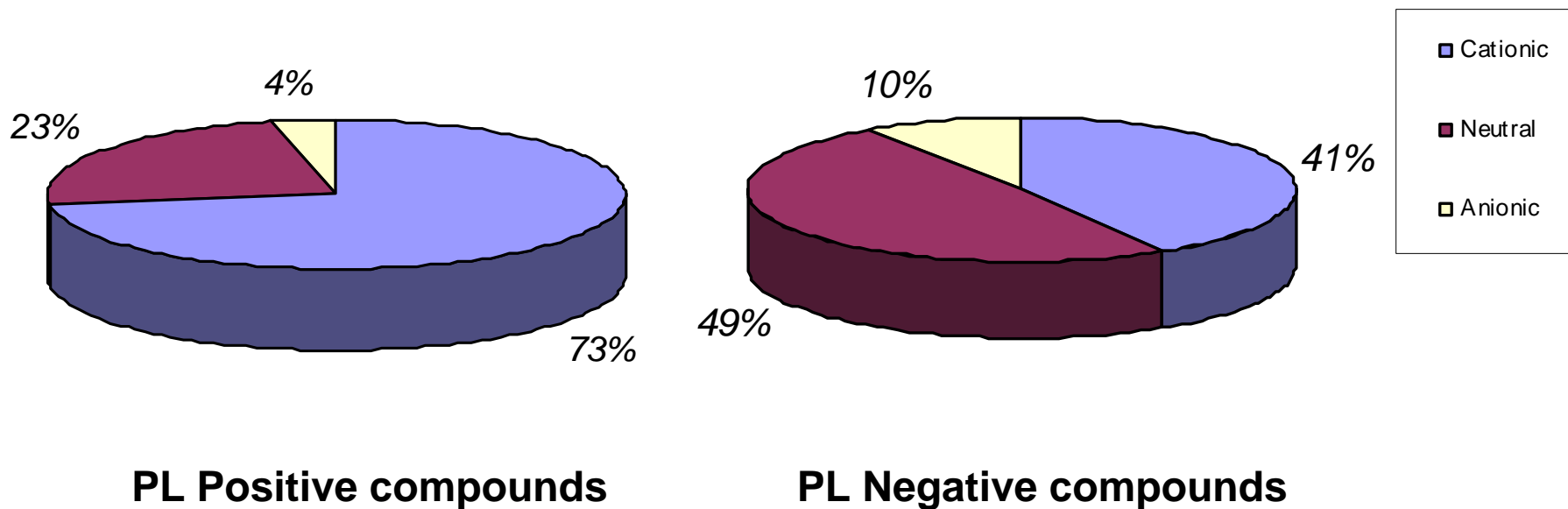
Validation Results for Ploemen and Pelletier Models



Pelletier, et al, *J. Chem. Inf. Model.* (2007) 47:1196-1205.

Ploemen, et al, *Experimental and Toxicologic Pathology.* (2004) 55:347-355

Physicochemical properties associated with PLD: Net Charge (at pH 4.7)



Overall (Q)SAR Predictive Performance

- Leadscope exhibited the highest concordance (79.4%) overall among the three models tested for predicting PLD
- MC4PC and MDL-QSAR models show higher sensitivity than previous CDER models by Kruhlak, et al. (2008)
- Derek for Windows had low sensitivity, but high positive predictivity (81.6% of those predicted positive were correctly identified)

Limitations of PLD Models

- Although negative compounds in the enhanced database are considered higher confidence than in previous investigations, very few were evaluated with electron microscopy
- Models do not account for drug metabolism, dose, duration of exposure, or species specificity
- Models only predict PLD induction, not resulting toxicity
- Chemical structure-activity relationships and physicochemical parameters may not be entirely predictive of PLD - PLD may be induced by other mechanisms
- None of the models consider 3D molecular attributes

Focus of CDER laboratory research on PLD

#1 – Potential biomarkers that could help guide clinical development of drugs with preclinical findings of PLD

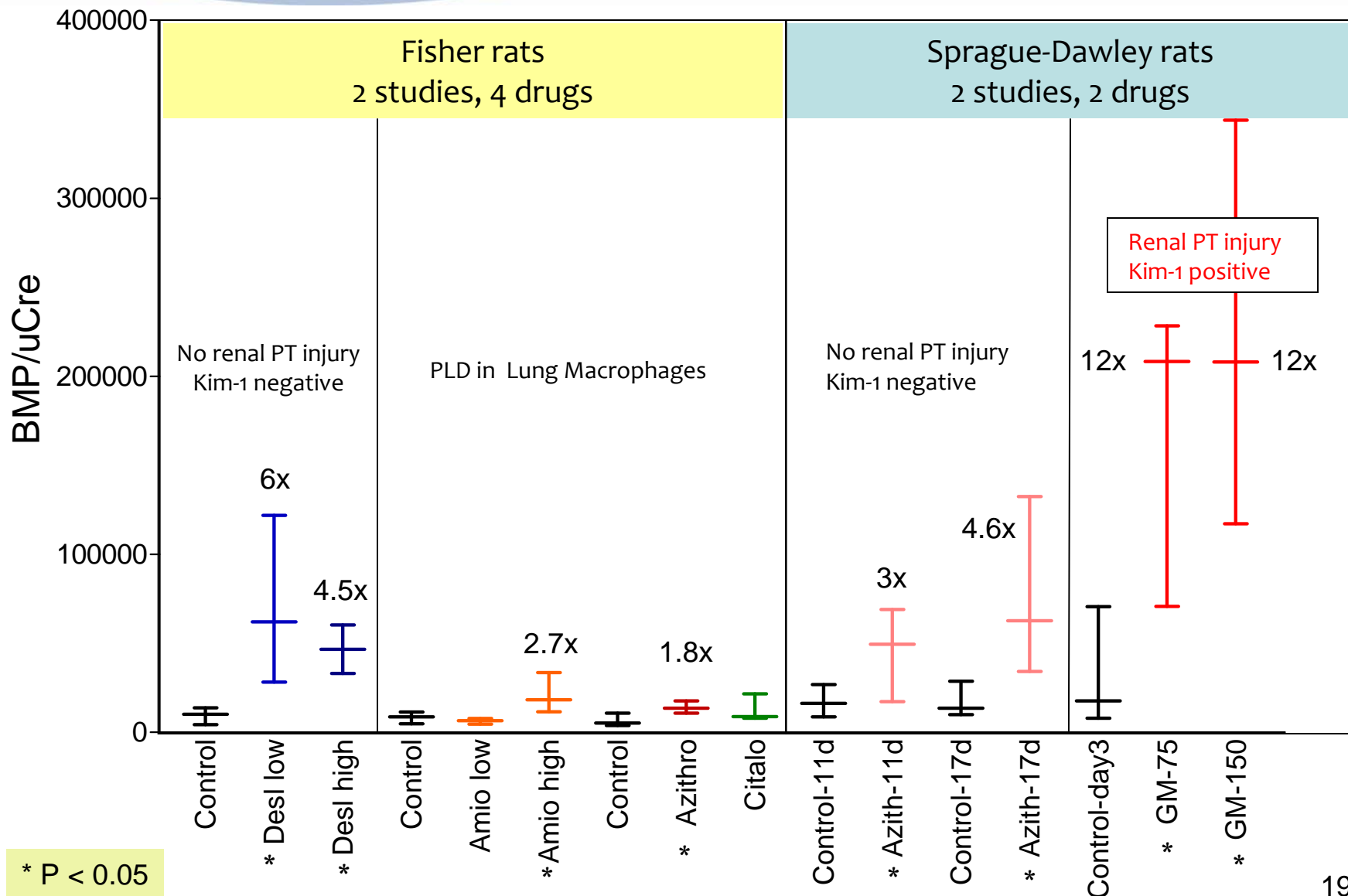
- Bis(monoacyl)glycerol phosphate (BMP) levels in urine or serum as a non-invasive marker of PLD

#2 - Improved methods for verification of PLD in preclinical studies for regulatory risk assessments

- Lamp-2 (lysosome-associated membrane protein 2) immunostaining to assay tissue PLD

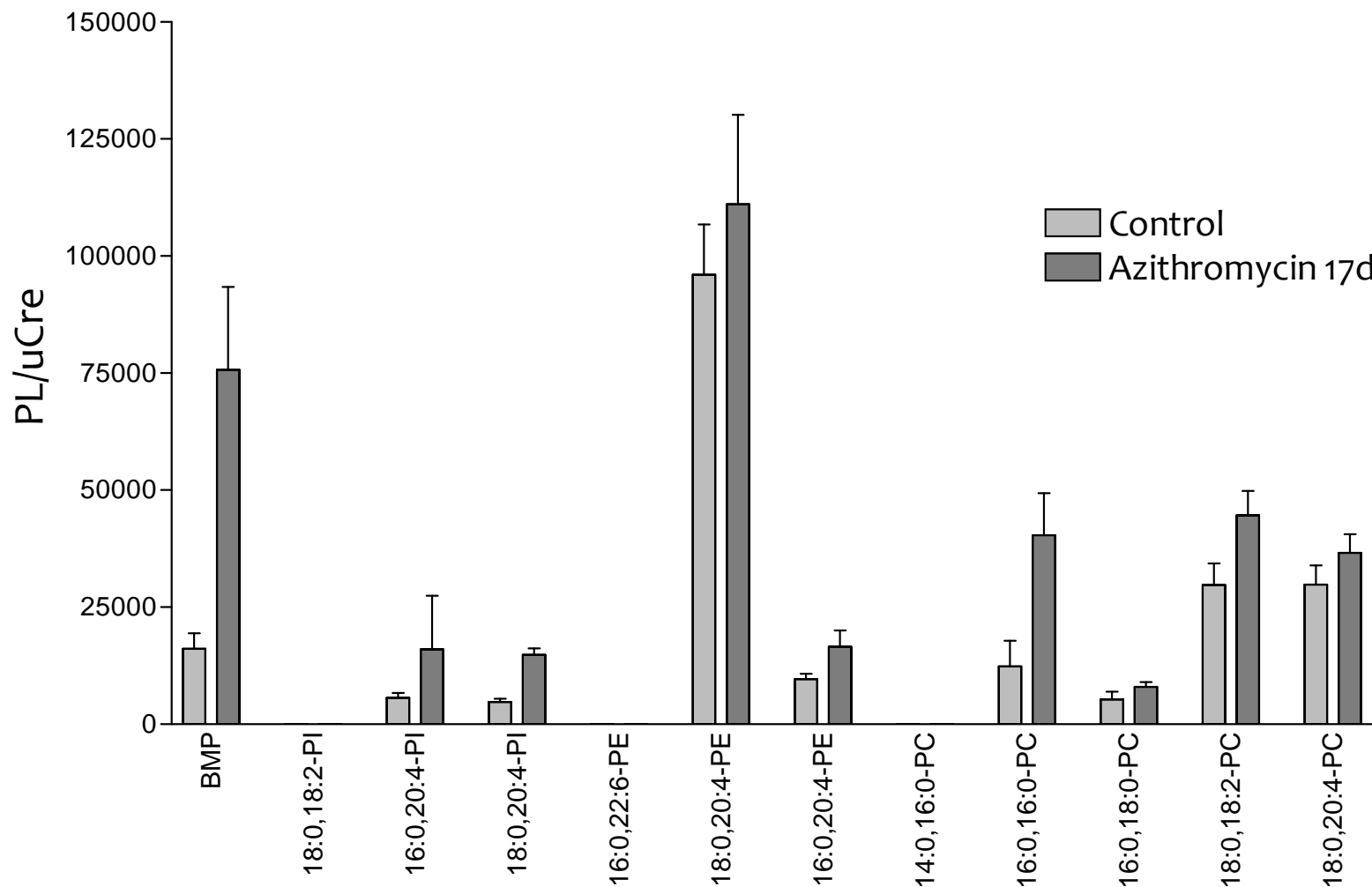
Summary of in-life studies conducted to test model systems for PLD assay evaluations

DRUG	RAT STRAIN	DOSE AND DURATION	REPORTED SITES OF PLD	OBSERVED SITES OF PLD (H&E)	OBSERVED SITES OF PLD (LAMP2 IHC)	SITES OF TOXICITY
Amiodarone	Fisher	30 or 60 mkd, 4 wks	Lung, liver, and kidney	Lung	Lung	None
Azithromycin	Fisher	100 mkd, 4 wks	Generalized	Lung	ND	None
Azithromycin	Sprague Dawley	150 mkd, 11 or 17 d	Generalized	No findings	Kidney	None
Citalopram	Fisher	100 mkd, 4 wks	Lung, lymph node, kidney	Lung	ND	None
Desloratadine	Fisher	40 or 60 mkd, 18 d	Lung, liver, kidney, pancreas	No findings	Kidney	None
Gentamicin	Sprague Dawley	75 or 150 mkd x 3 d +/- recovery period up to 2 weeks	Renal proximal tubules	ND	ND	Renal proximal tubules

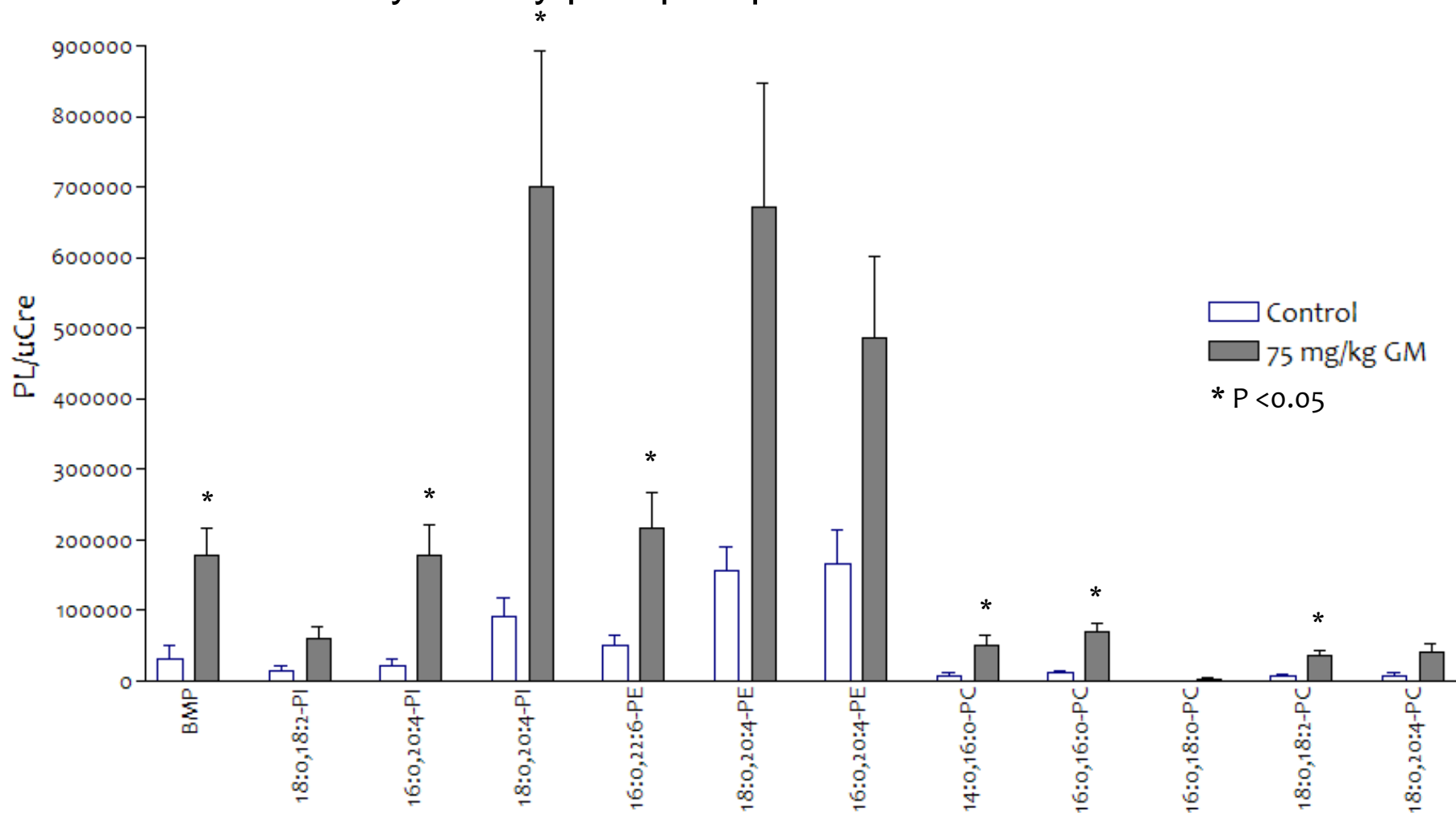


Statistically significant increases in urinary BMP were observed in most of our studies with known phospholipidotic drugs

In studies of phospholipidotic compounds without toxicity, significant changes in urinary phospholipids tended to be selective for BMP



In studies of phospholipidotic compounds with toxicity, significant changes were seen in many urinary phospholipids and were not selective for BMP



Conclusions on results-to-date on new biomarkers for phospholipidosis

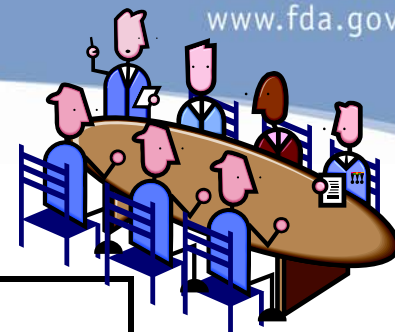
- Increases in urinary levels of BMP were induced by daily dosing for 2-4 weeks with known phospholipidotic drugs, while few consistent changes were seen in urinary levels of other phospholipids (PI, PE, PC).
- Increases in BMP and increased Lamp-2 IHC staining occurred prior to visualization of vacuolation at the light microscopic level, which is a first tier screen for phospholipidosis in tissues.
- Increases in urinary levels of BMP and most of the other 11 measured phospholipids were seen in a model of acute kidney injury. Phospholipiduria has been shown by Nykjaer et al. (2010) to be a marker of gentamicin-induced renal injury
- **In these studies, urinary BMP was specifically elevated in studies with PLD inducers without renal injury and nonspecifically elevated in studies with renal injury**

Summary

- Database of Phospholipidosis (PLD) has been created
- Strong correlation between PLD LOEL and Toxicological LOEL
- Species differences in tissue distribution
- Association between non-clinical finding of phospholipidosis and clinical QT prolongation
- Development of models predicting PLD (with ICSAS and CRADA)
- Lab research underway on evaluating BMP as a non-invasive biomarker and LAMP-2 as a tissue marker of PLD

Conclusions

- Drug-Induced Phospholipidosis potentially effects complex processes in cellular functioning involving phagocytosis, pinocytosis, vesicle fusion and release, membrane and protein recycling and transport, and intra-cellular pH control. PLD has been known to induce apoptosis or cellular necrosis.
- Great need to determine why and/or how PLD is associated with increasing toxicity in many compounds (e.g. Torsades de Pointes)



The End!

FDA – PLWG	
Fang Cai	Sydney Choi
Tom Colatsky	Thomas Flynn
Martha Garcia	Joe Hanig (co-chair)
Neil Hartman	Kylie Haskins
Karen Hicks	John Koerner
Naomi Kruhlak	Laurie McLeod-Flynn
Owen McMaster	Yanli Ouyang
Luqi Pei	Nakissa Sadrieh
Larry Sancilio (co-chair)	Belay Tesfamariam
Karol Thompson	Jim Weaver

Thanks to Scott Pine and Esther Kim for help with data graphics and Amabel Orogo For QSAR Modeling.



Phospholipidosis: Industry Perspective

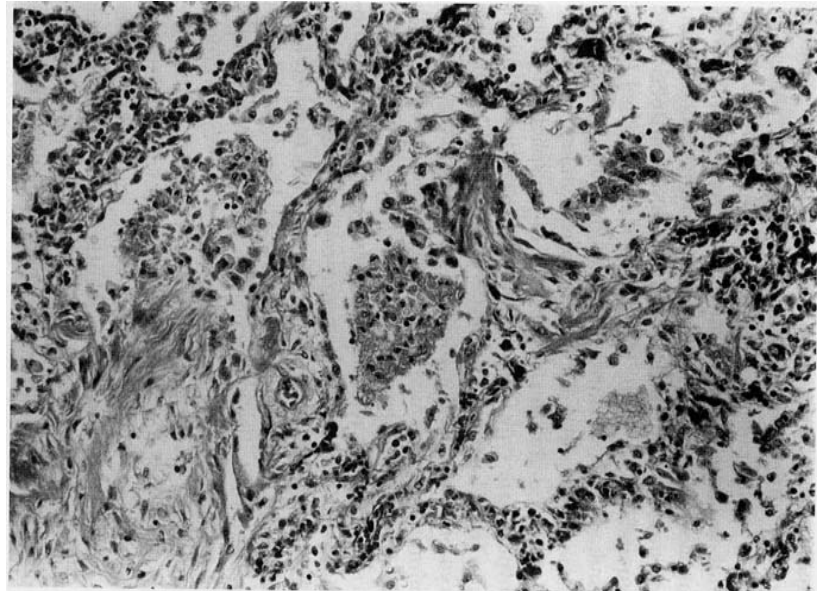
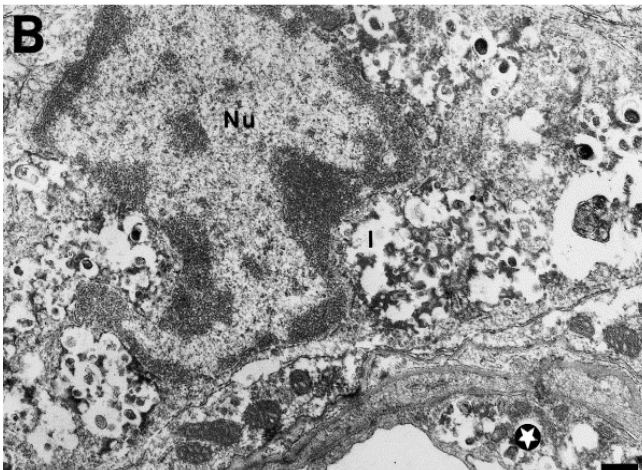
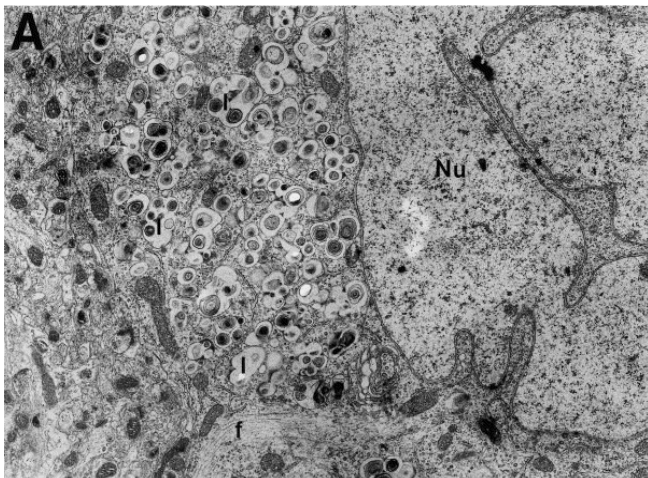
Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Vice-President for Regulatory Policy
sanofi-aventis

PL: A Persistent Problem in Drug Development

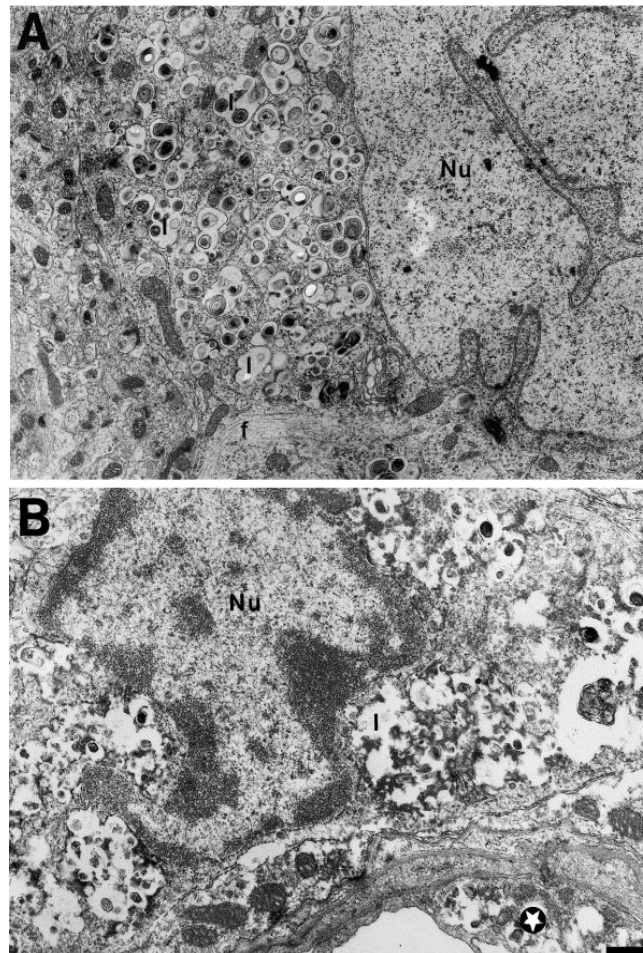
- Is PL an adverse effect?
- Is PL an “adaptive” response?
- Are there different types of PL, some “bad”, some not “bad”?
- Can PL be detected in clinical trials?
- Is/are there a useful biomarker(s) for PL?
- If there are different types of PL, are there methods for early detection (discovery toxicology) that allow for discrimination (esp. in the context of high-throughput combinatorial chemistry)?
- Biomarker(s) for PL versus PL-associated toxicity



So How Similar Are These Effects?



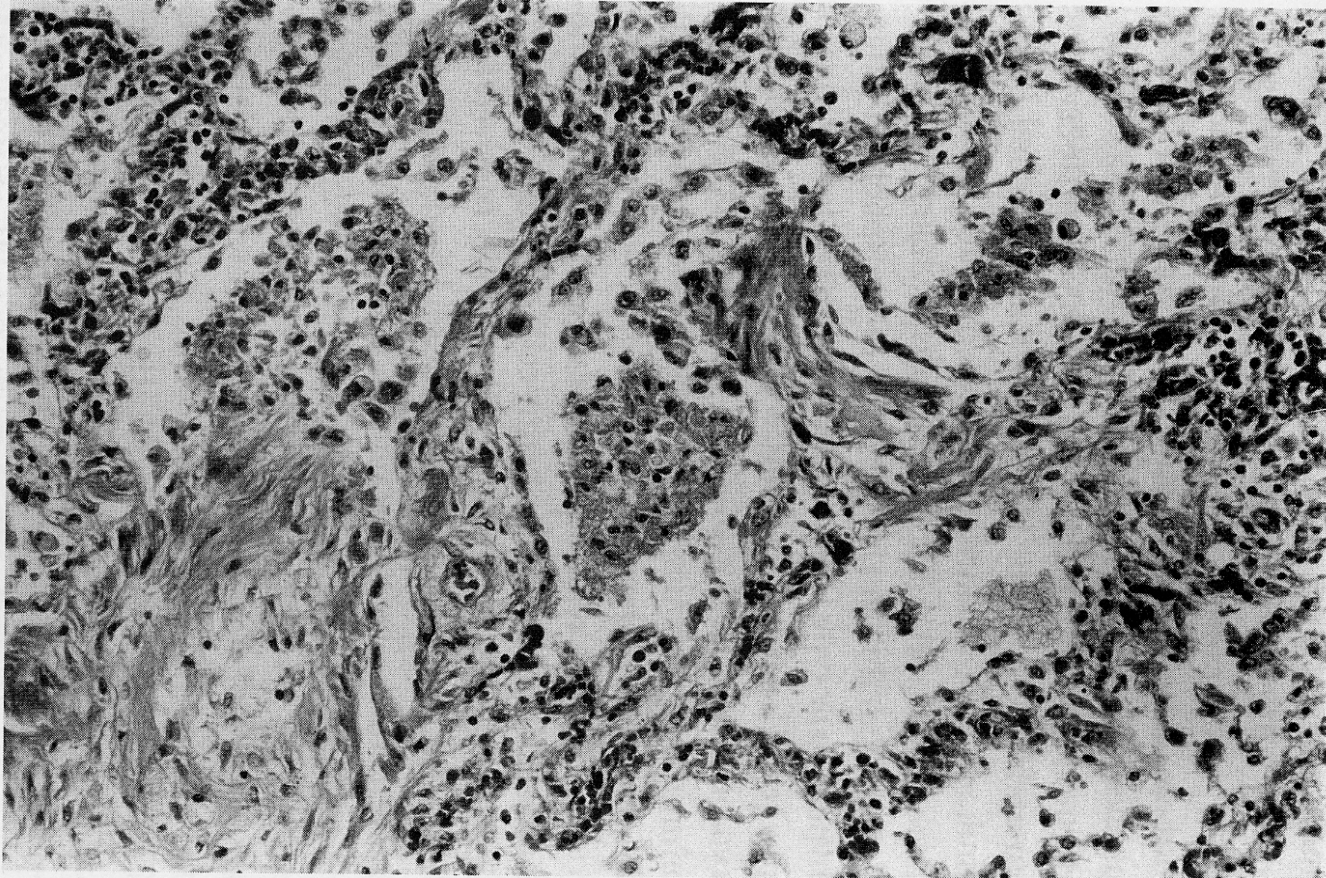
Mouse Model of Niemann-Pick Disease (Type C)



German et al., *Neuroscience* 109, 437-450, 2002



Amiodorone-Treated Rat Lung



Bedrossian, et al., *Ann. Diagn Pathol* 1: 47-56, 1997



“Niemann-Pick-like Syndrome”

- Old terminology for chemically-induced phospholipidosis

- Niemann-Pick disease(s): genetic diseases, Types A and B appear to be sphingomyelinase deficiency, Type C due to mutant gene encoding a 1278-amino acid protein, NPC1, that shares considerable homology with other proteins known to be important in regulating cholesterol balance across cells (e.g., sterol regulatory element-binding protein and 3-hydroxy-3-methylglutaryl coenzyme A reductase)



Important Points to Consider

- Chemically-induced phospholipidosis *may* involve enzyme inhibition, but effect likely temporary if involved at all
- Niemann-Pick Disease, as well as other inherited diseases, present with obvious pathology due to some sort of enzyme deficiency



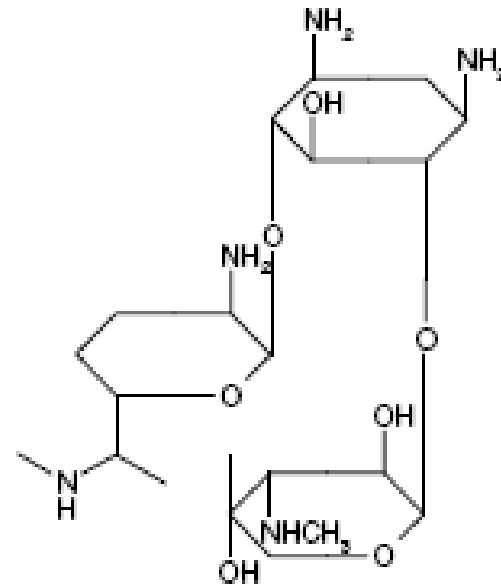
Regulatory Perspective

- Treat as adverse effect unless demonstrate otherwise, *knowing these possibilities*
- Physiologic response to CAD accumulation not associated with pathology
- Detoxification mechanism
- Simple depot effect
- Pharmacodynamic effect (concentration in target tissue)



Is There a Compelling Example?

- Maybe gentamicin
- Not typical CAD
- PL in renal tissue (esp, proximal tubule)
- Inhibition of PL inhibits renal toxicity
- However, questions remain





Is there a need for guidance?

- **Phospholipidosis is a common finding**
- **Unfortunately, no current consensus on “concern level” of finding**
- **Lack of consistency in dealing with the issue: tendency to re-invent approach with each new drug**



Potential Guidance

- If phospholipidosis is observed in non-clinical studies, is there an association with pathology (not likely, but first consideration)?
- Is there an association with functional effects in affected tissues (e.g. QT prolongation)?
- Does the effect appear to intensify over time, and is there any PK association?



Potential Guidance (2)

- What are the tissue types affected, and are these known to be targets of phospholipidosis-associated pathology(ies)?
- Is the effect reversible?
- Is the effect associated with PD?
- Can biomarkers of effect be identified?
- Can nonclinical/clinical methods be recommended?



Biomarkers

- May offer tool to enable clinical trials *without* understanding mechanism or safety implications
- Depends on sensitivity
- Also, pattern of biomarker may be informative (phospholipiduria: BMP only → PL, toxicity unknown; BMP + other phospholipids → greater concern, although kidney only at present)
- Lack of phospholipiduria sufficient to exclude clinical relevance of PL findings in toxicology studies?



Clinical QT Prolongation

- Thorough QT clinical study usually expected (ICH E14), thus PL would be a further signal to support need for evaluation
- Mechanistic basis for QT effect
- However: lack of better hERG concordance needs exploration (other mechanism for PL-QT association not captured by assay?)
- Use of biomarker in conjunction with PK for risk management?



Computational Toxicology

- Attractive issue since SAR known (CAD \rightarrow PL)
- New methods very good at predicting effect
- However: metabolism remains issue
- Are there models that can predict biotransformation of non-CAD to CAD metabolite?
- Care with results: some CADs are valuable therapeutics: reliance on SAR could limit pool of candidate drugs without justification
- Are there toxiphores that can be useful in candidate selection where CAD structure is essential for desired activity?



Genomics and Systems Biology

- Use of biomarkers, SAR, etc., can enable drug development without understanding of PL effects?
- Ultimately, need to determine enough about PL safety issues to make informed decisions
- Simply finding PL in toxicology studies not sufficient
- Genomic markers have given some insight into affected biologic pathways, may offer clue to distinguishing “bad” from “not bad” PL



Conclusions

- PL is a problem in drug development
- It will not go away if ignored
- Assumptions about “adaptive response” versus “toxicity” should be supported by experimental evidence
- Excellent example of in-house FDA Critical Path effort to deal with persistent problem