# Pharmacokinetics and Efficacy of Encochleated Atovaquone (CATQ) in Murine Model of *Pneumocystis*

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# DISCLOSURE

I do not have a financial interest, arrangement or affiliation with a commercial organization that may have a material interest in the subject matter of my presentation

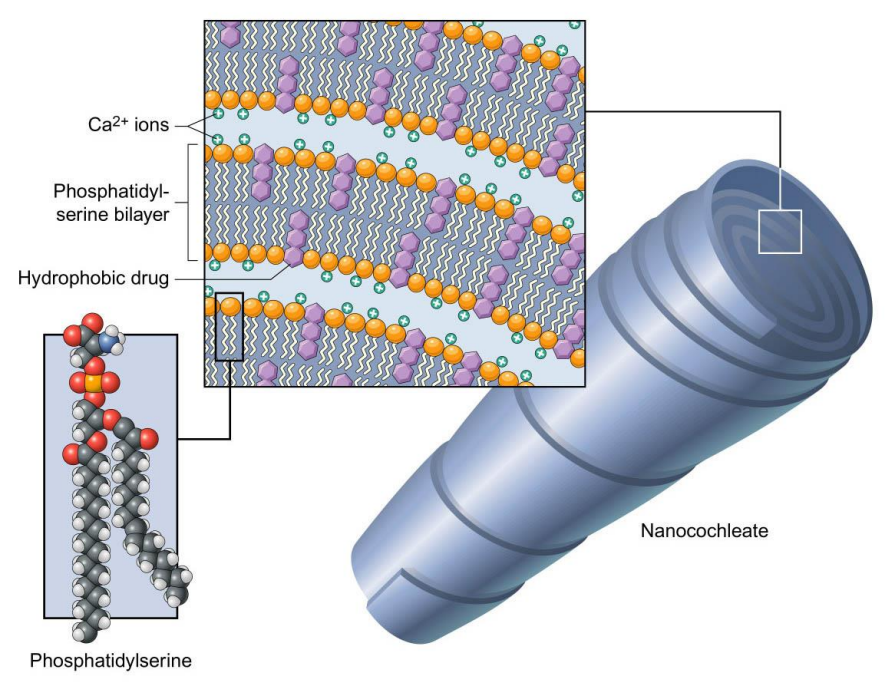
# ATOVAQUONE FOR TREATING *PNEUMOCYSTIS* PNEUMONIA (PCP) AND *TOXOPLASMA GONDII*

- Alternative agent for treatment and prophylaxis of PCP and toxoplasmosis if unable to tolerate trimethoprim/sulfamethoxazole or sulfadiazine
  - High rate of AEs (20-85%): Rash (including SJS and TENS), fever, hepatotoxicity, bone marrow suppression (transplant)[1]
  - Up to 36% of patients experience DLT→ discontinuation of TMP-SMX[2]
- Poor tolerability of current commercially available formulations
  - Poor taste/palatability, nausea, diarrhea, rash, headache and transaminase elevations

# ATOVAQUONE PK PROPERTIES[3,4]

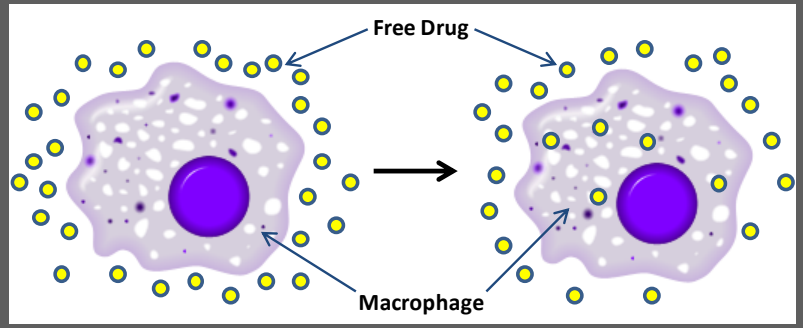
- Highly lipophilic, Vd= 0.6 L/kg
- Protein bound (99.9%)
- Long terminal half life at SS (2-3 days)
- Saturable absorption/non-linear PK
  - Absolute F= 47%, ↑ 2-3-X with high fat meal
  - Therapeutic response and mortality correlated with plasma concentrations in both PCP (≥ 15 mcg/mL) and toxoplasmosis (≥18.5 mcg/mL)[5-7]

# STRUCTURE OF ENCOCHLEATED ATOVAQUONE (CATQ)



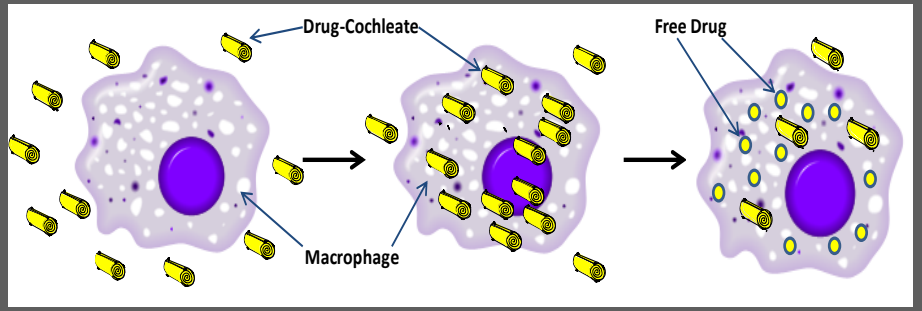
# COCHLEATES CHANGE THE PHARMACOKINETICS AND BIODISTRIBUTION OF DRUGS

## Traditional Model of Drug Delivery




- High plasma levels→ relatively low intracellular levels
  - Higher doses required→ non-specific toxicity

## Cochleate Model of Drug Delivery – The "Trojan Horse" Hypothesis




- CATQ phagocytosis via MPS→ ↓ intracellular $Ca^{2+}$→ cochleates release ATQ
- Lower plasma levels required for equivalent efficacy→ less systemic toxicity

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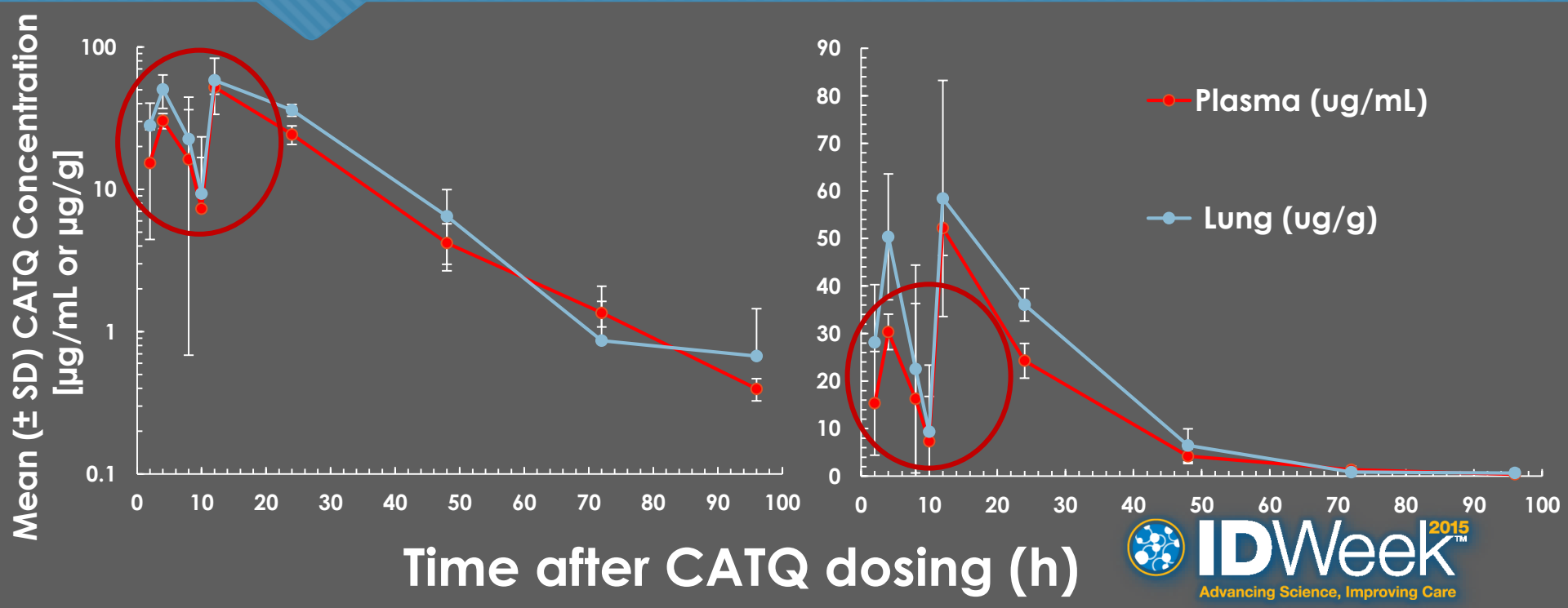
# PK STUDY DESIGN

- Murine PCP model (Univ. of Cincinnati)
  - Mice infected by cohousing with a *P. murina* infected mouse and immunosuppressed by addition of 4 µg/ml of Dexamethasone to the drinking water X 5 weeks
- CATQ (100 mg/kg) single dose administered via oral gavage
- Three mice sacrificed at ten time points: 0 (baseline), 2, 4, 8, 12, 24, 48, 72, and 96 hrs post-dose
- Blood and lung samples collected at each time
- PK parameters calculated by Non-Compartmental methods via Phoenix WinNonlin (v 6.4, Certara, St. Louis, MO)

# CATQ CONCENTRATIONS IN PLASMA AND LUNG TISSUE FOLLOWING 100MG/KG ORAL DOSE

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# PK RESULTS IN PLASMA AND LUNGS

| Pharmacokinetic parameters | Plasma | Lung | Ratio (Lung:Plasma) |
|---|---|---|---|
| **Cmax** (ug/mL) or (ug/mg) | 52.4 | 61.7 | 1.18 |
| **AUC all** (hr*ug/mL) or (hr*ug/mg) | 1142 | 1648 | 1.44 |
| **Half-life** (hr) | 12.4 | 14.8 | 1.20 |
| **Tmax** (hr) | 12 | 12 | - |
| **Volume of distribution (Vd/F)** (mL/kg) or (mg/kg) | 1551 | - | - |
| **Clearance (CL/F)** (mL/hr/kg) or (mg/hr/kg) | 87.0 | 59.7 | 0.69 |

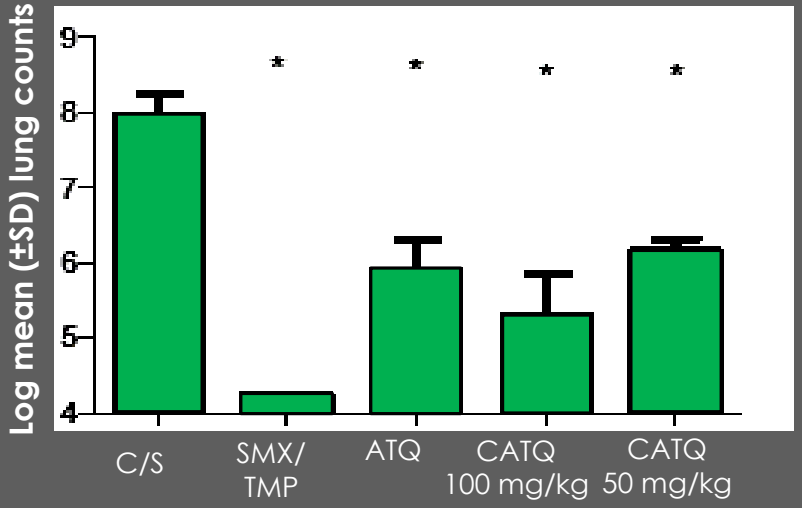
# PNEUMOCYSTIS EFFICACY STUDY DESIGN

- Mice infected same method as PK study
- 21 days of treatment, n= 8-10 per group
- Lungs processed for microscopic enumeration of asci and nuclei
- Comparisons between groups performed by one-way ANOVA followed by the Tukey's multiple comparison test and student's t test when appropriate.

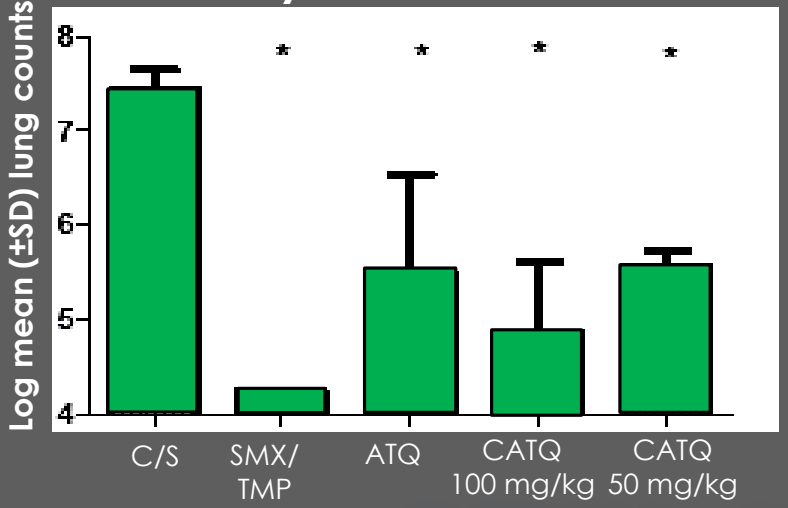
| Group | Drug and Dose |
|---|---|
| 1 | Vehicle treated negative control |
| 2 | SMX-TMP treated positive control (250/50 mg/kg) |
| 3 | Atovaquone treated control (100 mg/kg) |
| 4 | CATQ High dose (100 mg/kg) |
| 5 | CATQ Low dose (50 mg/kg) |

# DAY 21 PNEUMOCYSTIS BURDEN

## Nuclei Counts




## Cysts Counts




* p< 0.05 vs C/S

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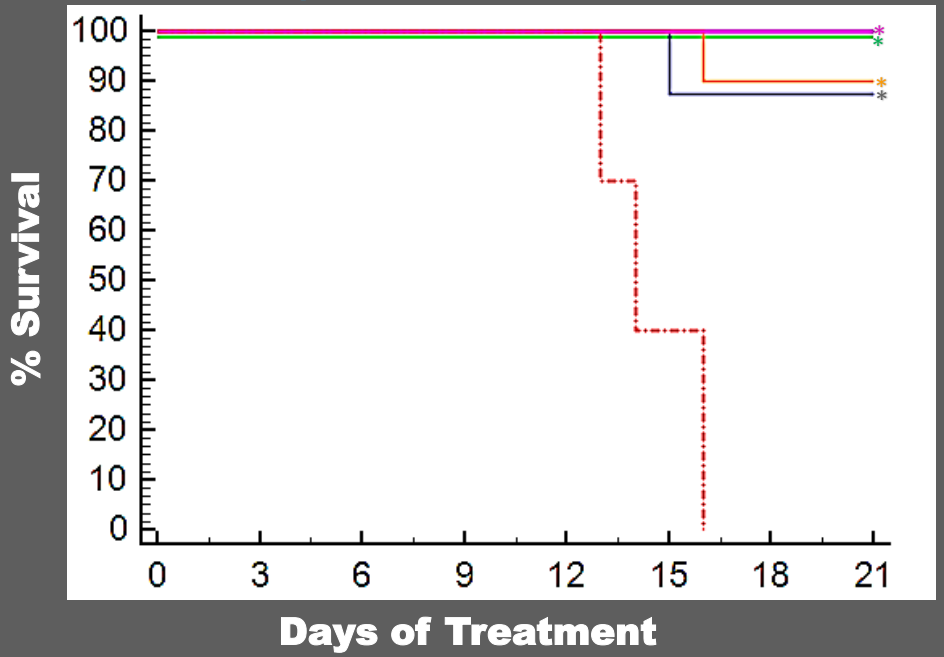
# SURVIVAL AFTER 21 DAYS OF TREATMENT




ATQ 100 mg/kg
CATQ 100 mg/kg
CATQ 50 mg/kg
Control (C/S)
SMX/TMP 250/50 mg/kg

* p<0.05 vs Control (C/S)

# CONCLUSION/NEXT STEPS

- CATQ represents a viable potential therapeutic candidate for treatment of PCP
  - PK and biodistribution favorable with long half life and relatively higher exposure in lungs than plasma
  - Dose-dependent efficacy observed in the treatment study with equivalent efficacy at ½ the dose of the formulation of atovaquone already approved for the treatment of PCP
- Future studies are warranted

# CONTRIBUTIONS


- **Authors/Collaborators:** Parag Kumar, PharmD[1], Melanie Cushion, Ph.D.[2,3], Michael Linke, PhD[2,3], Ruying Lu, BS[4], Pankaj Desai, PhD[5], Ganesh Moorthy, M.Pharm[5], Raphael Mannino, PhD[6], Chris Lambros, PhD[7], Edmund Tramont, MD, FIDSA[7], Larry Sallans, PhD[8], J. Carl Craft, MD[4], Thomas Sesterhenn, MS[2], Alan Ashbaugh, BS[2], Margaret Collins, BS[2], Keeley Lynch, BS[2], Susan Bonitz, PhD[4] and Joseph Kovacs, MD[9],
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# REFERENCES


1. HIV OI guidelines
2. *Ann Intern Med.* 1996;124(9):792-802
3. Mepron/malarone package insert
4. CROI ATQ abstract
5. 19
6. 20
7. 21

# Appendix (methods)

- PK study sample preparation for analysis:
  - Plasma: Samples were extracted using 3 ml ethyl acetate as extraction solvent. To 100 µl of the plasma sample, 25 µl of internal standard (4 ng/mL ATQ-d4) was added and vortexed. After mixing, 3 ml of ethyl acetate was added and the samples were vortexed for 30 seconds. The samples were then centrifuged for 5 minutes. The supernatant was transferred to another test tube and evaporated to dryness using a centrifugal evaporator. The residue was reconstituted in 100 µl acetonitrile: water (80:20) solution, mixed, and transferred to mass spec vials.
  - Lung: Sample weight was noted and homogenate was prepared using 1mL PBS buffer and a tissue homogenizer. Further lung samples were extracted using a similar extraction method as plasma with ethyl acetate as the extraction solvent. To 100 µl of the lung sample, 25 µl of internal standard (4 ng/mL ATQ-d4) was added and vortexed. After mixing, 3 ml of ethyl acetate was added and the samples were vortexed for 30 seconds. The samples were then centrifuged for 5 minutes. The supernatant was transferred to another test tube and evaporated to dryness using a centrifugal evaporator. The residue was reconstituted in 100 µl acetonitrile: water (80:20) solution, mixed, and transferred to mass spec vials.

# Appendix (methods)

- Lung Nuclei and Asci enumeration and processing:
  - The entire lung was dissociated in 10 mL of PBS by means of a gentleMACS Dissociator instrument (Miltenyi Biotec, Auburn, CA). The lung tissue wass then filtered through a 40 µm mesh, and P. murina recovered by centrifugation at 2000 × g for 5 min. Erythrocytes werere lysed with aqueous ammonium chloride (0.85%), washed, centrifuged and resuspended in 1 ml of phosphate buffered saline. Three 0.01 drops each covering an area of 1 $cm^2$ were placed on a pre-etched glass slide and air-dried. The slides were stained with cresyl echt violet (CEV), which selectively stains P. murina asci, and Diff-Quik (DQ), a rapid variant of the Wright Giemsa stain that stains the nuclei of all developmental stages. The slides werere coded, read in a blinded manner, and the number of asci or nuclei per oil immersion field (OIF) were determined by randomly counting 30 OIF (10/drop). This number was multiplied by a conversion factor for the microscope and by the dilution to arrive at the total number per lung. The lower limit of detection of microscope in detecting P. murina in mice was log10 4.35 (2.23 x 104) organisms/lung.