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Efficacy of Oral Cochleate-Amphotericin B for the Prevention of Invasive Candidiasis in Neutropenic Mice.

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INTRODUCTION

Introduction: Amphotericin B (AMB), due to its fungicidal efficacy, broad spectrum and limited resistance, can be considered the "gold standard" antifungal treatment and remains the principal therapeutic option for deep mycoses. However, its application is currently limited by toxicity and administration requiring slow intravenous injection.

MAT2203, (AMB cochleates; CAMB), a novel lipid nano-crystal formulation of AMB, demonstrates oral bioavailability, significant efficacy, low toxicity, and shelf-life stability.

In animal models, CAMB demonstrates antifungal activity with similar efficacy as intraperitoneal AMB deoxycholate, without the associated toxicity. Oral administration of CAMB has shown strong efficacy in mouse models of cryptococcal meningitis, disseminated candidiasis and disseminated aspergillosis.

In a Phase 2a human clinical study being conducted at the NIH Clinical Center, under the direction of Dr. Alexandra Freeman, MAT2203 (CAMB) has shown efficacy, safety, and tolerability in predominantly hereditary immunodeficient patients with a recurrent or chronic mucocutaneous candidiasis infection (esophageal, oropharyngeal, vaginal) who are refractory or intolerant to standard non-intravenous therapies.

Background: MAT2203 (CAMB) is being developed for the prevention of invasive fungal infections due to immunosuppressive therapy, particularly in patients with acute lymphoblastic leukemia (ALL). In patients being treated for ALL the risk for invasive fungal infections (IFIs) is high, with an associated high risk of lethality. Currently, there is no standard of care for preventing these high risk IFIs in ALL patients.

The established treatment regimens for ALL are highly sensitive to liver-metabolized drug-drug interactions, causing serious concerns for drug-drug interaction induced side-effects.

Amphotericin B is not liver metabolized and when incorporated in the lipid-crystal nano-particle structure of MAT2203, (CAMB) this otherwise toxic IV only compound can now be safely orally administered, providing patient convenience over ~12 weeks prophylactic treatment duration, without the typical kidney and liver toxicity associated with other Amphotericin B formulations.

Purpose: This study evaluated the efficacy of orally delivered CAMB for the prevention of invasive candidiasis caused by a virulent *C. albicans* WT strain SC5314 in immunocompromised, neutropenic mice.

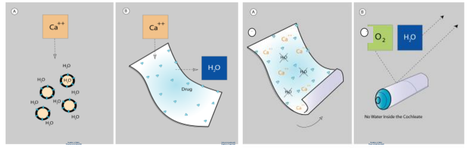
Results: CAMB was highly effective in preventing invasive candidiasis in neutropenic mice. CAMB at 5 mg/kg or above resulted in significantly increased survival, as well as organ sterilization, in contrast to the 100% mortality of untreated mice. Remarkably, survival corresponded with organ sterilization, as all but one survived mice showed sterilization in all four organs evaluated, regardless of dosage. In mice that died prior to the assessment time point, burden reduction efficacy of CAMB was dose-dependent in kidneys and lungs, but not in liver and spleen. No significant toxicity was observed with CAMB treatment by gross assessment.

Conclusion: At or above 5 mg/kg dosing level, CAMB is highly effective in preventing invasive candidiasis in neutropenic mice. Prophylactic and therapeutic treatment of CAMB at 5 mg/kg or above has resulted in significantly increased survival as well as organ sterilization, in contrast to the 100% mortality of untreated mice at early post-infection time point. Remarkably, survival corresponded with organ sterilization, as all but one survived mice had sterilization in all four organs evaluated, regardless of dosage. In mice that died prior to assessment time point, burden reduction efficacy of CAMB is dose-dependent in kidneys and lungs, but not in liver and spleen, indicating drug delivery to liver and spleen may not be as sufficient as that to kidneys and lung.

LIPID NANO-CRYSTAL ("LNC") PLATFORM DELIVERY TECHNOLOGY

How Cochleates Encapsulate Drugs

Our lipid nano-crystal ("LNC") platform delivery technology has been shown to mediate **oral bioavailability for injectable drugs**, **reduce toxicity**, and significantly **enhance intracellular drug delivery**. Lipid nano-crystals are stable nano-particles composed of simple, naturally occurring materials: phosphatidylserine and calcium. They have a unique multilayered structure consisting of a large, continuous, solid, lipid bilayer sheet rolled up in a spiral or as stacked sheets, with no internal aqueous space. This unique structure provides protection from degradation for "encochleated" molecules. Cargo molecules within the interior of the cochleate remain intact, even though the outer layers of the cochleate may be exposed to harsh environmental conditions or enzymes.



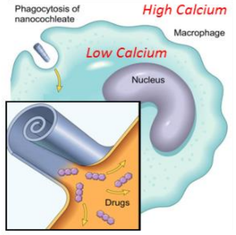
Formation of Stable Drug-Liposome Intermediate | Calcium Interaction with Negatively Charged Lipid | Formation of Stable Drug-Cochleate Nano-Crystal

- The API is associated with the negatively charged lipid.
- The addition of calcium creates a calcium-phospholipid anhydrous crystal.
- Nano-crystals are composed of layers of a lipid-calcium complex.
- The API is trapped in or between the layers protecting the API from harmful environmental elements.

Cell-Targeted Delivery

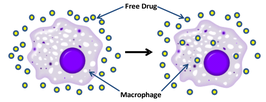
- **Macrophage readily engulf our lipid nano-crystals and their cargo**
- **Once inside the macrophage, the low level of calcium in the cytoplasm causes the nano-crystals to open, releasing the cargo molecule**

Divalent cation concentrations *in vivo* in serum and mucosal secretions are such that the cochleate structure is maintained. Hence, the majority of cochleate associated molecules are present in the inner layers of a solid, stable, impermeable structure. Once within the interior of a cell, however, the low calcium concentration results in the opening of the cochleate crystal and release of the entrapped API.



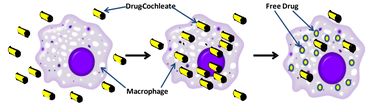
Our LNC Platform Technology Can Change the Pharmacokinetics and Biodistribution of Drugs

Traditional Model of Drug Delivery



- **Free drug in the extracellular milieu must cross the cell membrane in order to be effective against intracellular microorganisms.**
- **High plasma and interstitial drug levels are needed.**
- **A relatively low percentage of circulating drug enters the cell.**
- **Drugs with these properties have difficulty treating intracellular infections.**
- **High circulating drug levels can result in nonspecific toxicity.**

Model of Cochleate Mediated Drug Delivery The "Trojan Horse Hypothesis"



- **High calcium concentrations in gastrointestinal secretions, serum and interstitial fluid stabilize the drug cochleate crystal.**
- **Drug lipid nano-crystals enter the circulatory system, diffuse into tissues and/or are taken up by "activated" and/or infected cells.**
- **Intracellular levels of drug lipid nano-crystals increase and reach high levels.**
- **The low intracellular calcium concentration causes the drug lipid nano-crystals to open releasing their cargo molecules.**
- **Lower plasma levels are required to reach efficacious intracellular drug concentrations.**
- **These lower plasma levels may result in less systemic toxicity.**

CAMB PREVENTION OF INVASIVE CANDIDIASIS IN NEUTROPENIC MICE

Murine infection model and antifungal treatment.

A neutropenic mouse model of invasive candidiasis was used in this study.

Animals. Female 8-week-old BALB/c mice weighing ~20g were used for this experiment. Mice were housed in presterilized filter-top cages and maintained in accordance with American Association for Accreditation of Laboratory Care criteria. The animal study was approved by Rutgers Institutional Animal Care and Use Committee.

Strain. *C. albicans* susceptible strain SC5314 were subcultured in liquid yeast extract-peptone-dextrose (YPD) medium at 37°C with shaking overnight. Cells were collected by centrifugation, washed twice with sterile phosphate-buffered saline (PBS), and counted with a hemocytometer. *Infection dose:* The infection dose was 5.04×10^4 CFU per mouse.

Drug. CAMB/MAT2203 (0.25, 0.5, and 1 mg/ml) and placebo cochleates were provided by Matinas.

Experiment Methods. Mice were rendered neutropenic by receiving 150 mg/kg and 100 mg/kg of cyclophosphamide via IP injection on day -4 and day -1 prior to infection, respectively. Groups of 10 mice were randomized into 6 treatment arms. CAMB at 2.5, 5, or 10 mg/kg, or placebo control was administered once daily via oral gavage (p.o.), starting from day -3 until day 4. On day 0, mice were infected with ~ 5×10^4 CFU of *C. albicans* SC5314 via retro-orbital injection. Mice were monitored for signs of illness and those became moribund before the scheduled time point were euthanized humanely. All survived mice were sacrificed via CO_2 inhalation at day 5 post-infection. Kidneys, lungs, liver, and spleen were aseptically removed from all mice in the study. All remaining organs were homogenized in 5 ml of sterile PBS, and 100 µl of homogenate or proper dilutions were spread onto YPD agar plates for fungal burden counts.

Treatment	No. of survivors at sacrifice	Log_{10} CFU/g of tissue (sterilization %)			
		Kidney	Lung	Liver	Spleen
Placebo	0	6.90±0.19	6.66±0.42	5.75±0.18	6.08±0.52
CAMB 2.5 mg/kg	2	5.47±2.20* (20%)	5.27±2.12* (20%)	5.74±2.66 (20%)	5.03±2.02 (20%)
CAMB 5 mg/kg	7	4.48±2.24*** (70%)	4.08±1.98*** (60%)	5.32±2.67 (70%)	5.08±2.55** (70%)
CAMB 10 mg/kg	10	0**** (100%)	0**** (100%)	0**** (100%)	0**** (100%)

Relative to placebo *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001

Results: A number of deaths was reported on day 2 post-infection, with 10, 7, and 2 mice from the placebo, CAMB 2.5 mg/kg, and CAMB 5 mg/kg groups, respectively. One mouse from the CAMB 2.5 mg/kg group and 1 from the 5mg/kg group turned moribund on day 3 post-infection and had to be euthanized. All remaining mice survived through the endpoint, hence, the 5-day survival was 0, 20%, 70%, and 100% for placebo, 2.5 mg/kg CAMB, 5 mg/kg CAMB, and 10 mg/kg CAMB, respectively. The average fungal burden in placebo treated mice was observed as 6.9 log10 CFU/g in kidneys, 6.7 log10 CFU/g in lungs, 5.8 log10 CFU/g in liver, and 6.1 log10 CFU/g in spleen (**Table**). In comparison, mice treated with CAMB demonstrated a dose-dependent survival and organ sterilization response. The 2.5 mg/kg therapy resulted in 20% survival, with complete sterilization of all organs in survived mice, and 1.5, 1.4, and 1 log burden reduction in kidneys, lungs, and spleen in dead mice relative to placebo mice. Liver fungal burden in dead mice in the 2.5 mg/kg group was not different from those treated with placebo. Similarly, the 5 mg/kg regimen protected 7 of 10 mice from death and complete sterilization was achieved in all organs but one lung sample from all survived mice. As for the dead mice treated with 5 mg/kg CAMB, kidney and lung burdens dropped ~ 1 log compared to those died in the 2.5 mg/kg group, while liver and spleen burdens were not much different. The 10 mg/kg CAMB treatment was the most efficacious with 100% survival and organ sterilization.

Conclusion: At or above the 5 mg/kg dosing level, CAMB is highly effective in preventing invasive candidiasis in neutropenic mice. Prophylactic and therapeutic treatment of CAMB at 5 mg/kg or above has resulted in significantly increased survival as well as organ sterilization, in contrast to the 100% mortality of untreated mice at early post-infection time point. Remarkably, survival corresponded with organ sterilization, as all but one survived mice had sterilization in all four organs evaluated, regardless of dosage. In mice that died prior to assessment time point, burden reduction efficacy of CAMB is dose-dependent in kidneys and lungs, but not in liver and spleen, indicating drug delivery to liver and spleen may not be as sufficient as that to kidneys and lung.