Lipid-Crystal Nano-Particles: Formulation and Delivery of Oligonucleotides

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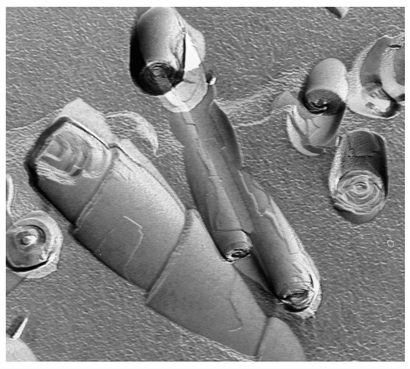
Summary: Lipid-Crystal Nanoparticles, Cochleates, have been shown to efficiently formulate and stabilize oligonucleotides. Oligonucleotide-cochleate formulations are stable on the shelf, in tissue culture, and in biological fluids. The unique and proprietary cochleate delivery mechanism protects the oligonucleotide from the external environment, concentrates it to the target tissue, and safely and efficaciously delivers the oligonucleotide into the target cell, while exhibiting extremely low toxicity. Importantly, cochleate anti-microbial formulations have been scaled-up to 100 liter, commercial size batches under GMP conditions, and the lead cochleate product, Amphotericin B-Cochleates, (CAMB), is entering phase 2 human clinical trials.

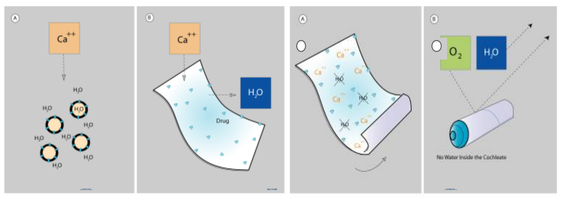
LIPID-CRYSTAL NANO-PARTICLE COCHLEATE PLATFORM TECHNOLOGY



- Stable, phospholipid-calcium crystals
- Self-assembling crystalline units
- Multilayered structure, containing little or no internal aqueous space
- Increases shelf life stability
- Resistant to degradation in GI tract
- Inexpensive cost of goods and manufacturing
- Phase IIA Human Clinical Efficacy Trials (NIAID Clinical Center)

COCHLEATE TECHNOLOGY –FORMULATION AND DELIVERY STRATEGY

How Cochleates Encapsulate Drugs

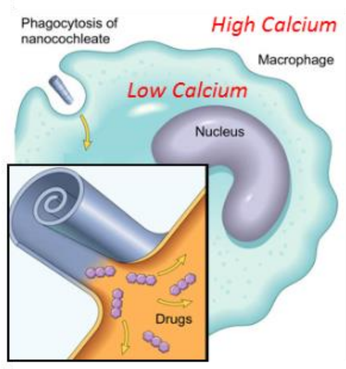


Formation of Stable Drug-Liposome Intermediate | Calcium Interaction with Negatively Charged Lipid | Formation of Stable Drug-Cochleate Nano-Crystal

Cochleate delivery vehicles have been shown to mediate **oral bioavailability for injectable drugs**, **reduce toxicity**, and significantly **enhance intracellular drug delivery**. Cochleates are stable, lipid-crystal, 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. Components 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.

Cell-Targeted Delivery

- Activated cells, including macrophages and virally infected cells, readily engulf cochleates and their cargo
- Once inside the macrophage, the low level of calcium in the cytoplasm causes the cochleate to open, releasing the cargo molecule

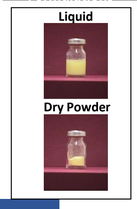


Divalent cation concentrations *in vivo* in serum and mucosal secretions are such that the cochleate structure is maintained. Hence, the majority of cochleate associated molecules are present in the inner layers of a solid, stable, impermeable structure. Once within the interior of a cell, however, the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

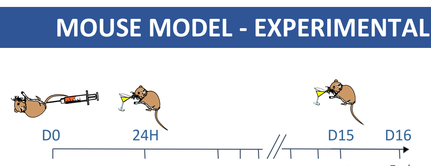
ORAL DELIVERY OF AMPHOTERICIN B COCHLEATES IS EFFICACIOUS AGAINST DISSEMINATED CANDIDIASIS

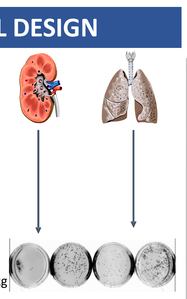
- Oral administration of AmB-cochleates has been shown to be as effective as equivalent, injectable doses of the leading AmB formulation (Fungizone) in mouse models of systemic candidiasis and aspergillosis.
- AmB-cochleates also demonstrate substantially lower toxicity than existing commercial AmB products.
- AmB-cochleates showed good safety in rats and dogs in 7 and 28 day toxicity studies.
- A commercially viable and cost effective manufacturing process for AmB-cochleates has been developed, and scaled-up 100 liter GMP batches of AmB-cochleates have been produced.
- An IND for AmB-cochleates is open, and data from a phase Ia human clinical trial were supportive of further studies.
- A phase 2a efficacy study is targeted to begin in Q2 2016.

CAMB Formulations



MOUSE MODEL - EXPERIMENTAL DESIGN



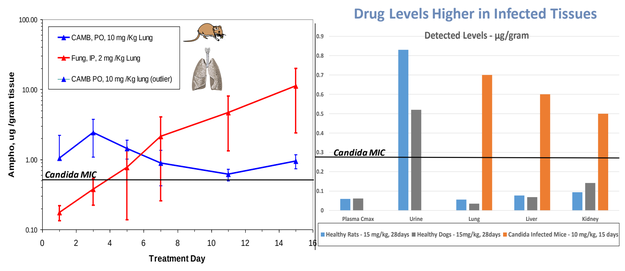


Mice:Balb/C, 25g, 10 mice/group
Infection: 1 x 10^6 CFU/ml, 0.2 ml/mouse, tail vein
Treatment: 15 daily doses in 0. 2ml of:
- AmB– Cochleate Intragastric at 0.5, 1.0, 2.5, 5.0 CAMBmg/kg
- AmBisome Intragastric at 10mg AmB/kg
- Fungizone IP 1mg AmB/kg, (Positive Control)
- Empty Cochleates, Intergalactic (Negative Control)

Tissue Burden: Kidneys and lungs in saline, serial 10x colony count dilutions

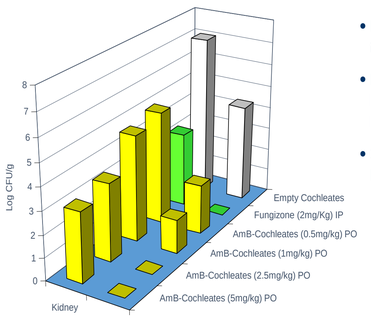
RESULTS

Targeting to Infected Tissue



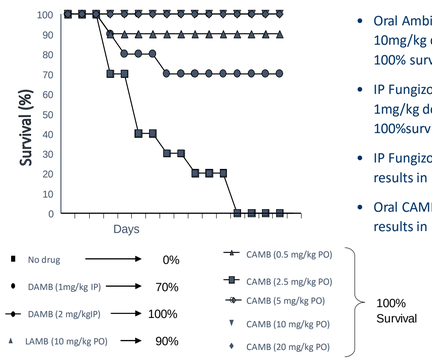
Oral CAMB Efficacy

Low Dose-Tissue Burden Study



- Low dose oral CAMB reduces tissue burden by 2 logs.
- High dose oral CAMB sterilizes lung and reduces tissue burden in kidneys by 4 logs.
- Oral CAMB efficacy similar to IP Fungizone.

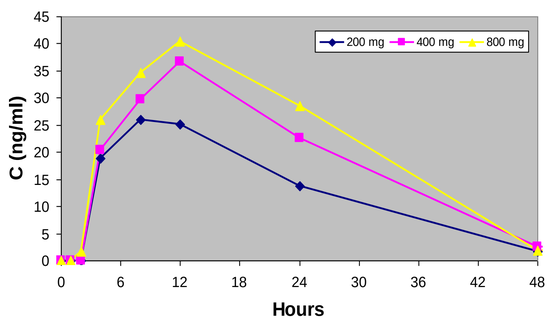
Survival



- Oral Ambisome (LAMB) at 10mg/kg does not result in 100% survival, (90%).
- IP Fungizone (DAMB) at 1mg/kg does not result in 100%survival, (70%).
- IP Fungizone at 2mg/kg results in 100% survival.
- Oral CAMB at 0.5mg/kg results in 100% survival.

ORAL DELIVERY OF CAMB – HUMAN PHASE I CLINICAL TRIAL: PHARMICOKINETICS

Oral Administration of CAMB in Humans: A Phase I Study of Tolerability and Pharmacokinetics



	200 mg	400 mg	800 mg
C_{max} (ng /ml)			
Mean	28.11	37.09	40.76
S D	17.06	8.66	6.48
Median	30.8	40.1	39.45
R ange	0 - 48.2	22.2 - 45.9	29.8 - 53.1
AUC_{0-24} (ng .h /ml)			
Mean	407.4	522.9	624.5
S D	302.3	274.0	294.9
Median	396.1	609.2	636.7
Range	0 - 853	0 - 816	0 - 1092
T_{max} (h)			
Median	8	12	12

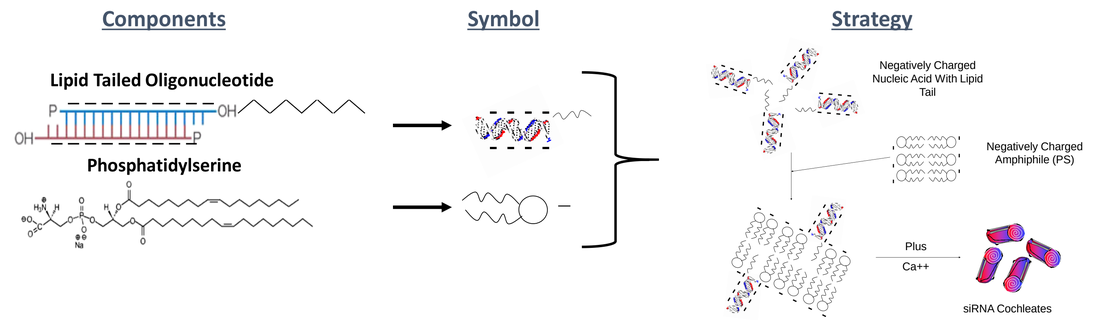
- Preliminary pharmacokinetic assessment indicated increases in C_{max} and AUC with increasing dose.
- Preliminary C_{max} and $AUC_{0\ 24}$ are compatible with prior results from animal toxicology studies.

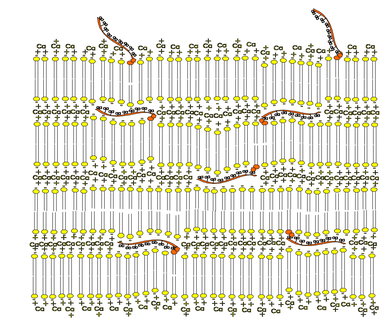
SUMMARY AND CONCLUSIONS

- In infected mice treated with oral CAMB at 10mg/kg, the liver, lung, and kidneys showed reproducible and quantifiable levels of AmB that achieved their maximal levels (~ 0.5-1 ug AmB/g tissue) early in the treatment schedule.
- Fungizone-treated mice showed lower tissue concentrations at early times, but nearly ten-fold higher accumulated AmB levels in liver and lung.
- Plasma AmB levels for oral CAMB were at or below the limit of quantitation (50ng/ml).
- Rapid tissue penetration to above MIC occurred in the infected organs of animals with invasive Candidiasis, while no such accumulation occurred in the organs of healthy animals, consistent with the targeted therapy concept.

Oral Administration of Drugs Formulated into Cochleates Can Change the Pharmacokinetics, Biodistribution and Tolerability of the Drug

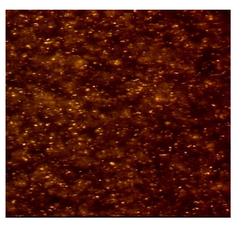
FORMULATION OF OLIGONUCLEOTIDES-LIPID TAILS STRATEGY



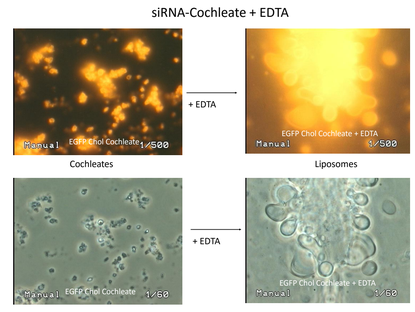


Lipid-Linked siRNA

Fluorescence microscopy of Cy3 labeled siRNA cochleates. Homogeneous preparation of particles < 1 micron.



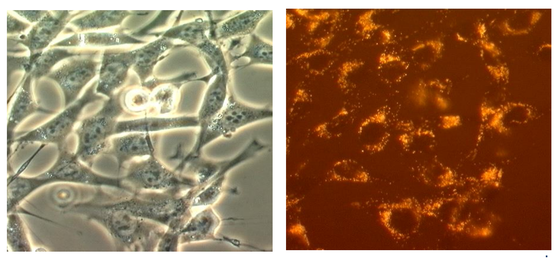
Cochleates Opening in a Low Calcium Environment



COCHLEATE DELIVERY OF OLIGONUCLEOTIDES

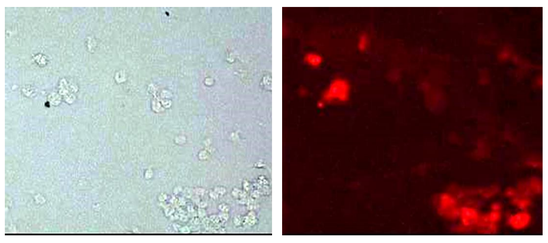
siRNA Nanocochleates – *In Vitro* Delivery

Light Microscopy | Fluorescence Microscopy



Cochleates deliver siRNA at high efficiency to every cell in the culture

In Vitro- Macrophages in Mouse Peritoneum

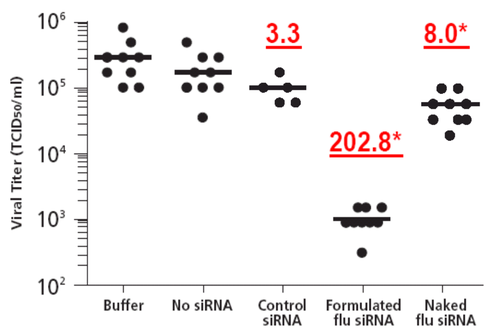


Rhodamine-labelled cochleate uptake by mouse macrophages *in vivo*. mouse macrophages isolated 6 hours after intra-peritoneal injection of 500µg rhodamine-labelled cochleates (25% rhodamine positive at 6 hours, 19% positive at 24 hours). 200x magnification

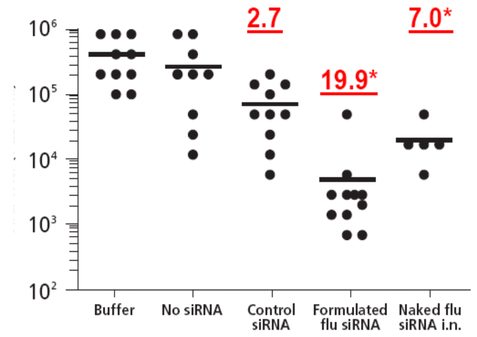
FORMULATION AND DELIVERY OF RNAi – INHIBITION OF INFLUENZA INFECTION

BALB/c mice were infected intranasally (100 pfu/mouse) with the PR8 serotype and then treated 5 hours later by either intranasal or intravenous administration of the indicated amounts of an influenza viral-specific siRNA and control siRNA in either cochleate siRNA formulation or non-formulated forms. The lungs were harvested 48 hours post-infection and viral titer was measured from lung homogenates by MDCK-HA assay.

Intranasal Administration of siRNA-Cochleates Inhibits Influenza Virus Production 200 Fold



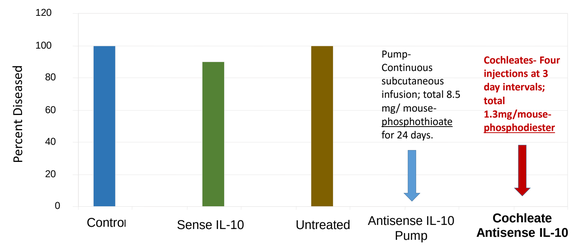
Intravenous Administration of siRNA-Cochleates Inhibits Influenza Virus Production 20 Fold



FORMULATION AND DELIVERY OF ANTISENSE DNA – MOUSE MODEL OF B CELL LYMPHOMA

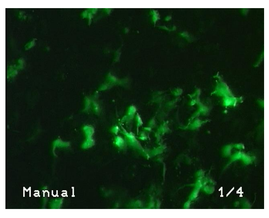
Results –

- **Encochleation protects the antisense DNA from degradation.**
- **Encochleated antisense DNA can be given by injection rather than continuous infusion.**
- **Encochleated antisense DNA is about 5 times for effective than free antisense DNA.**



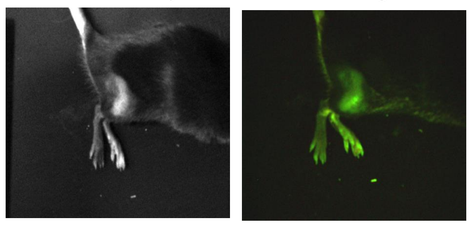
FORMULATION AND DELIVERY OF DNA PLASMID EXPRESSING GFP

In Vitro Delivery To Cultured Cells



DNA cochleates prepared by the trapping method were used to deliver a green fluorescent protein (gfp) expressing plasmid to SKOV3 cells. At least 75% of the cells were expressing gfp 72 hours after addition of cochleates.

In Vivo - *Injection into Mouse Leg*



Left panel: black and white image of c57 mouse after receiving intramuscular injection of 50uL gfp cochleates without casein and 50uL 4mM CaCl2 solution. Area injected (right leg) was shaved before injection.
Right panel: Image is same field under fluorescence. GFP signal is the sharp green in the middle of the shaved area, distinct from the light green haze that is background from the exposed skin. Picture was taken 72hrs after injection although fluorescent signal was seen 24hrs after injection also.

COCNCLUSIONS – COCHLEATE FORMULATION AND DELIVERY OF OLIGONUCLEOTIDES

- Cochleates have been used to formulate and stabilize a wide variety of biologically active molecules, including oligonucleotides.
- Stable formulations of encochleated oligonucleotides have been developed.
- Encochleation can achieve greater than 90% of the initial oligonucleotide present in end product.
- Cochleates protect oligonucleotides from degradation.
- Cochleate formulations are safe, non-toxic, and reduce toxicity.
- Cochleates are efficiently taken up by target cells and release the therapeutic molecule intracellularly.
- Encochleated oligonucleotide formulations deliver oligonucleotides into cells *in vitro* at high efficiency with no toxicity.
- Encochleated oligonucleotide formulations have shown activity *in vivo* in a mouse models.
- Cochleate formulations have been scaled-up using commercially viable manufacturing protocols.
- Anti-microbial cochleate formulations are in phase II human clinical trials.