Nano"Solutions" for Drug Delivery and Bioimaging

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San Francisco, CA

Mark Kester, Ph.D.

Director NanoSTAR Institute
University of Virginia

Conflict of Interest

- **Penn State Research Foundation has licensed ceramide nanoliposomes to Keystone Nano, Inc. (PA).**
- **Penn State Research Foundation has licensed calcium phosphosilicate nanoparticles to Keystone Nano, Inc. (PA).**
- **Penn State Research Foundation has licensed ORAL nanotechnology to Oraceutics, Inc. (VA)**
- **Penn State Research Foundation and UVA Innovation are negotiating a MOU to clarify joint intellectual property**
- **MK is co-founder and Chief Medical Officer of Keystone Nano, Inc. MK is co-founder of Oraceutics.**

What is Nano?

- 1 billionth of a meter
  - Human hair =80,000 nm
  - Atoms= 0.1-0.3nm
  - Wavelenths of visble light = 350-850nm
  - Human cell = 20,000 nm across
  - Single molecule of sugar = 2nm
- Defined as particles less than 100 nm in size
- National Nanotechnology Initiative
  - 3 billion US annually
- Technology at the atomic, molecular and macromolecular level
- Novel properties because of nanosize

- The future of medicine is small
  - Real small

- The future of medicine is small
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- The future of medicine is small
  - Real small

Desired Characteristics for a NanoParticle Drug Delivery Platform

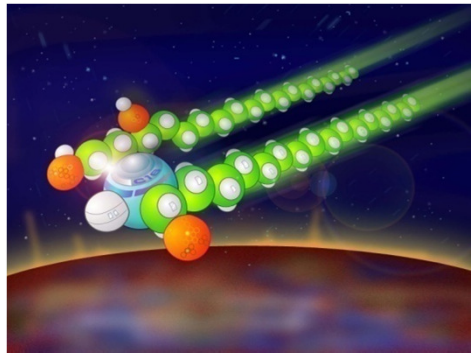
Desired Characteristic	Comments
Inherently non-toxic materials and degradation products	The initial material selection should be based on non-toxic materials especially with an aim toward human health care
Small Size (10 to 200nm)	There is not a particular size that seems most efficacious particularly based on in vivo studies. This is the range of particle diameters that have proven most effective for a wide variety of delivery systems. Also of note is the debate around the influence of particle shape.
Encapsulation of Active	To be effective, the active agent must be encapsulated within the nanoparticle vehicle. Surface decoration (i.e., adsorption) will often be effective *in vitro* but falls short for *in vivo* studies because of the reticuloendoplasmic systems in vivo
Colloidally stable in physiological conditions	The nanoparticle vehical and surface functionalization must resistant agglomeration for the solution pH values, ionic strength, macromolecular interactions, and temperature encountered in the physiological environment
Clearance Mechanism	The nanoparticle vehicle must have a ready clearance mechanism to avoid the cumulative and/or systemic effects of the drug laden particles
Long Clearance Times	Resistance to agglomeration and other effects that remove the nanoparticle encapsulated drug from the patient must be avoided to promote long circulation times in the circulatory system for as much of the nanoparticles to find and sequester in the cancer cells as possible
Biologically or extrinsically controlled release of therapeutic agents	There should be a trigger mechanism such as the acidic pH within the tumor or during endosome maturation designed into the nanoparticle platform to ensure the release of the encapsulated drug into the targeted tissue
Can be targeted to cell/tissue of choice	The nanoparticle platform should be able to be surface bioconjugated to target molecules for the specific cancer to provide the greatest uptake with the lesions and the least side effects with healthy tissue

From **Adair** et al., *ACS Nano*, 2010

Nanotechnology can turn Insoluble Drugs Soluble Ceramide as a Chemotherapeutic

Many chemotherapeutic apoptotic agents (anthracyclines, vinca alkaloids, antiestrogens, taxanes) promote endogenous ceramide accumulation

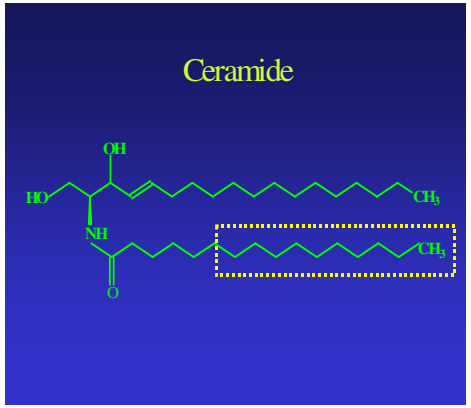
Exogenous ceramide treatment synergistically enhances Taxol- and Tamoxifen-induced tumor apoptosis



Ceramide is selectively apoptotic for transformed cells

However, systemic delivery of ceramide is limited by:

- **Cell impermeability**
- **Metabolism**
- **Precipitation**

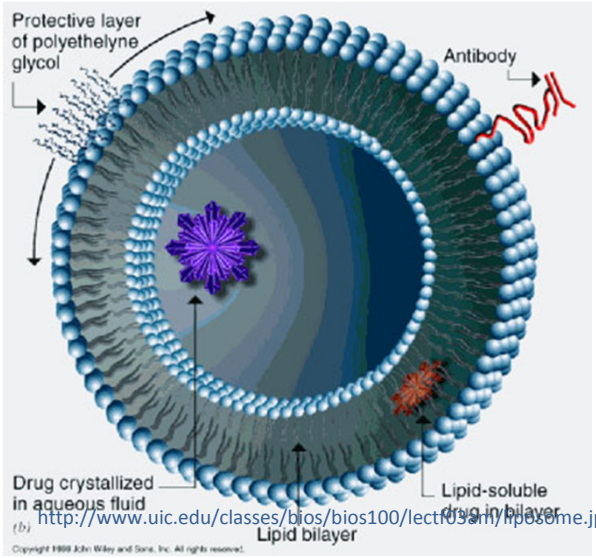


Therefore, suitable drug delivery systems are needed:

- **Nanoliposomes**
- **Nanocolloids**
- **Nanodendrimers**

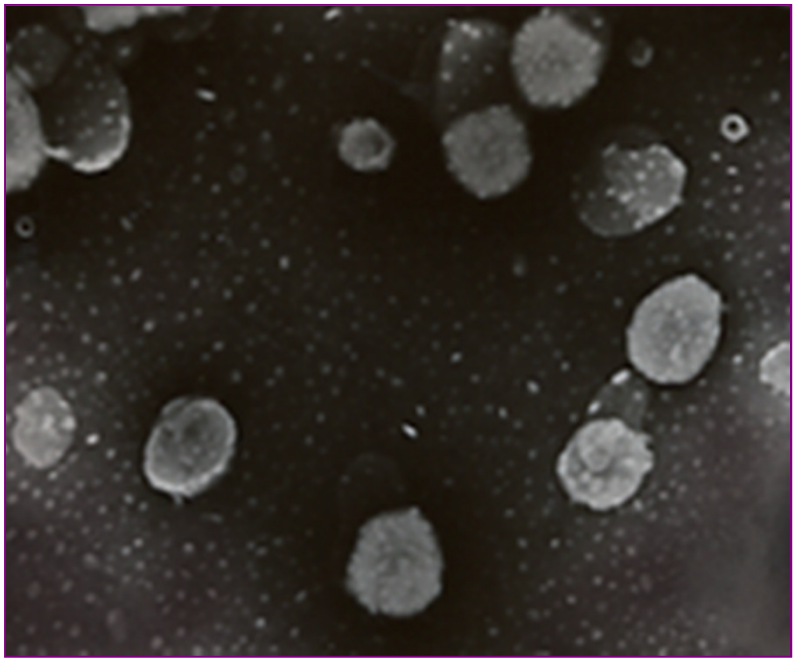
CNL-Based Drug Delivery

- The CNL is a stable, non-toxic nanoliposome formulation containing 15mol% PEG and 30mol% C_6-Ceramide.
- Hydrodynamic diameter = 87+/-10nm
- Zeta potential = -11+/-1mV
- Stable in biological fluids
- Shelf life of the CNL >6 months
- Increased solubility of Ceramide
- Enhanced and targeted delivery
- Protection from enzymatic degradation
- CNLs shown to inhibit cell proliferation and induce cell death *in vitro* and *in vivo* in solid tumor models, as well as leukemias

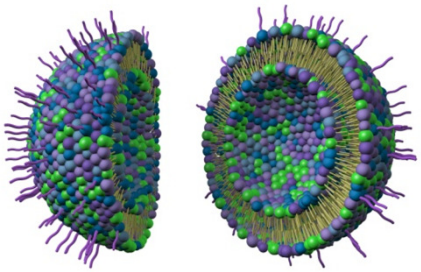


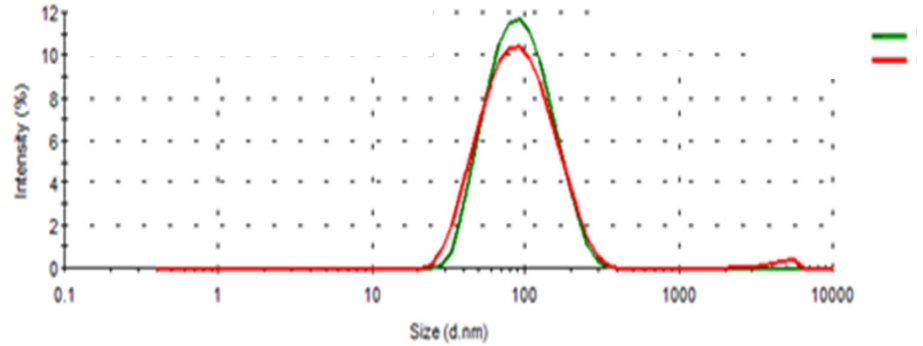
http://www.uic.edu/classes/bios/bios100/lectf03am/liposome.jpg

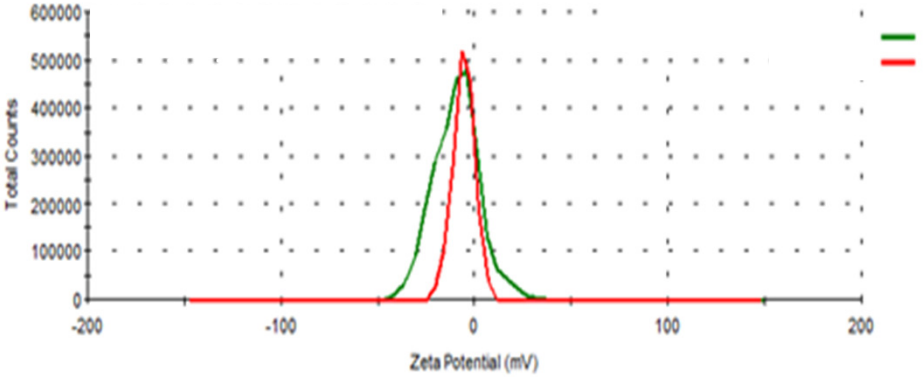
CNL
Morphology, Size Distribution & Zeta Potential



100 nm







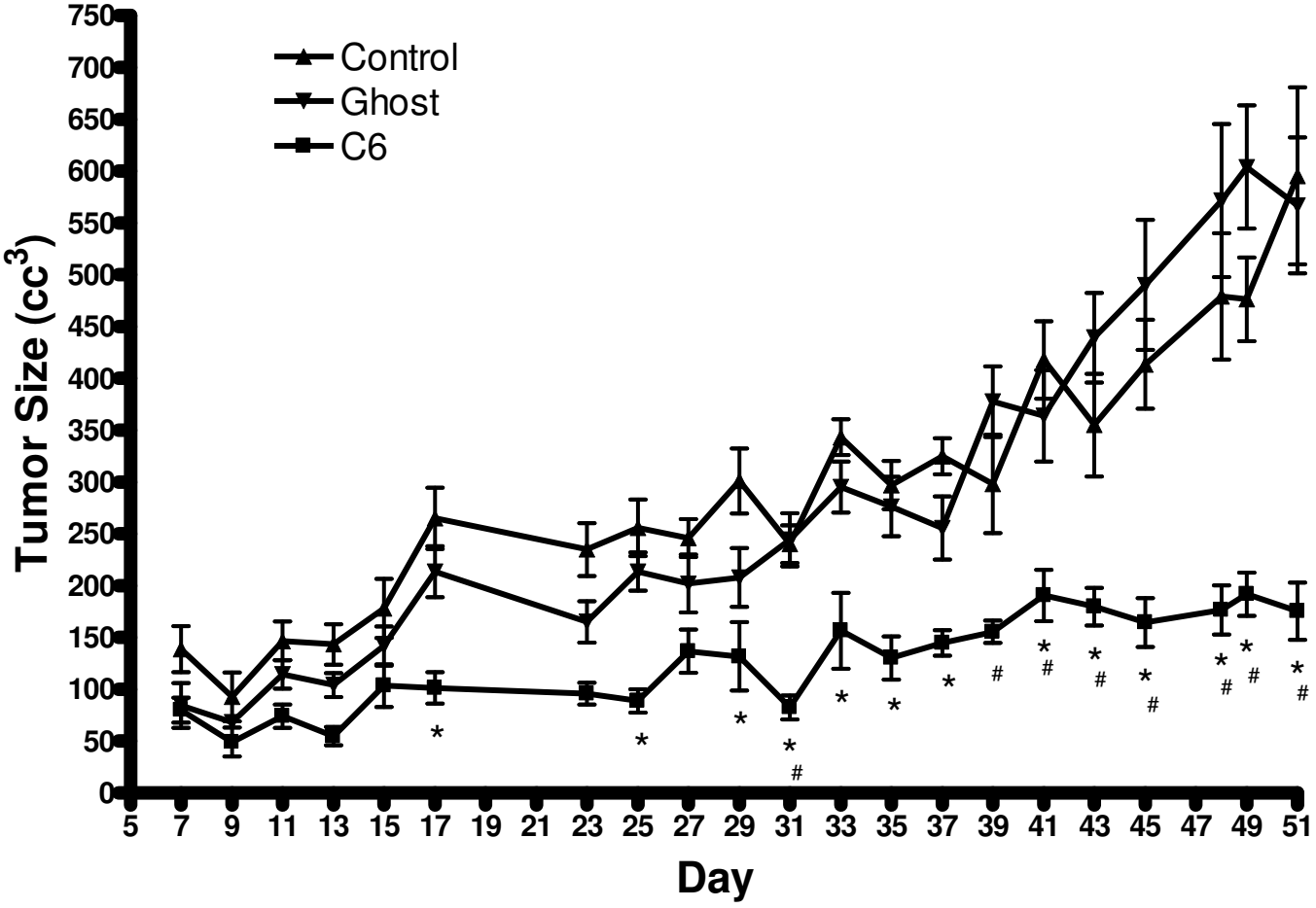
In vivo CNL Liposomal Delivery & Toxicology: Swiss Webster Mouse Model

- Liposomal formulation significantly reduces the LD_{50} of C_6 when administered IV in Swiss Webster mice
  - LD_{50} of "free", non-liposomal C_6 in DMSO vehicle was observed to be 10 mg/kg
  - No observable side effects of liposomal-C_6, Max dose tested = 200 mg/kg

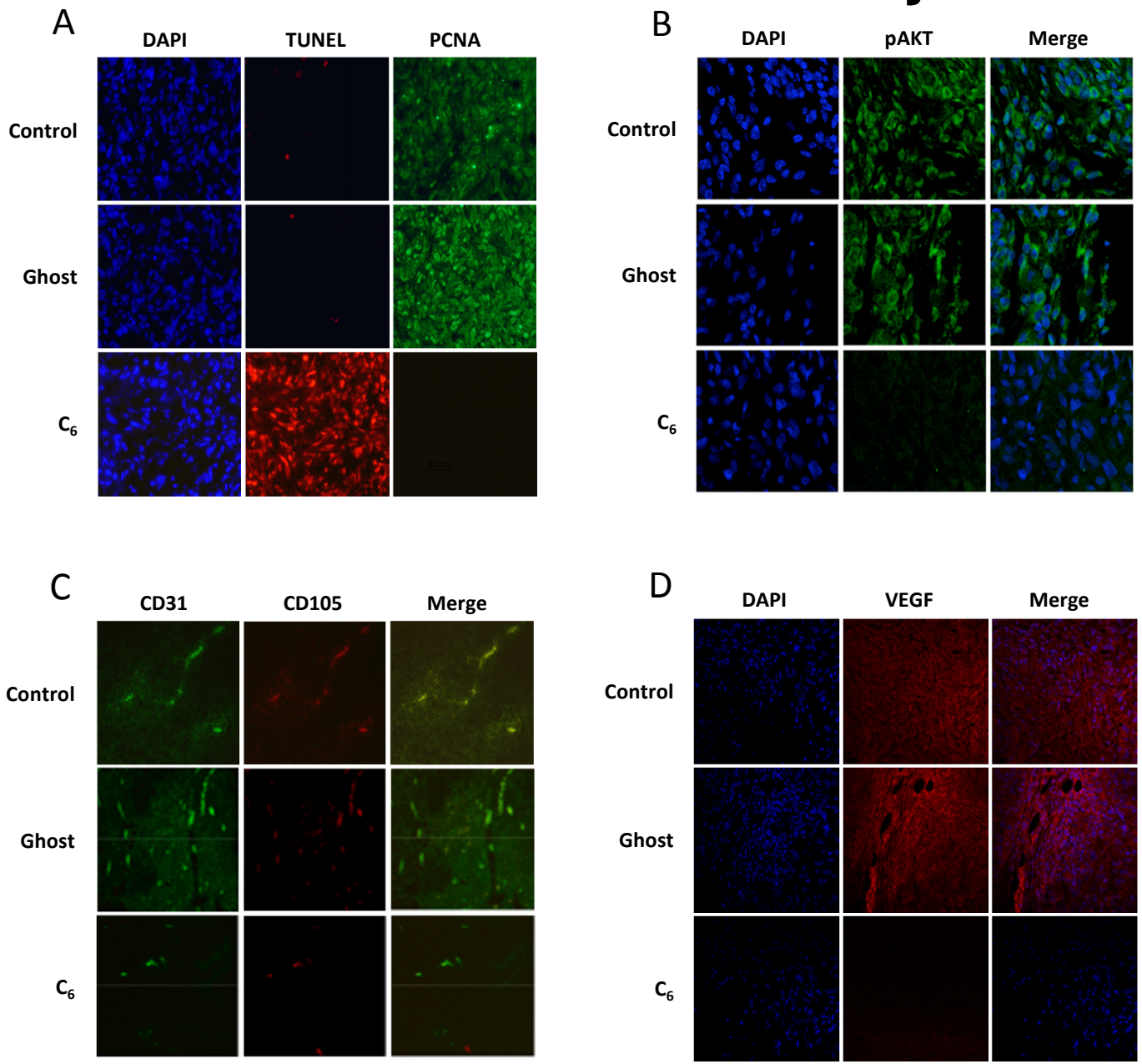
Hepatocellular Carcinoma

- **HCC Currently Untreatable**
  - Approximately 22,000 deaths per year in the US (60 per day)
  - Orphan Drug Candidate in US
  - Approximately 600,000 deaths worldwide per year from HCC (1,700 per day)
  - Patients typically die within 6 months of diagnosis
  - Current therapy – Sorafenib adds approximately 8 weeks to life

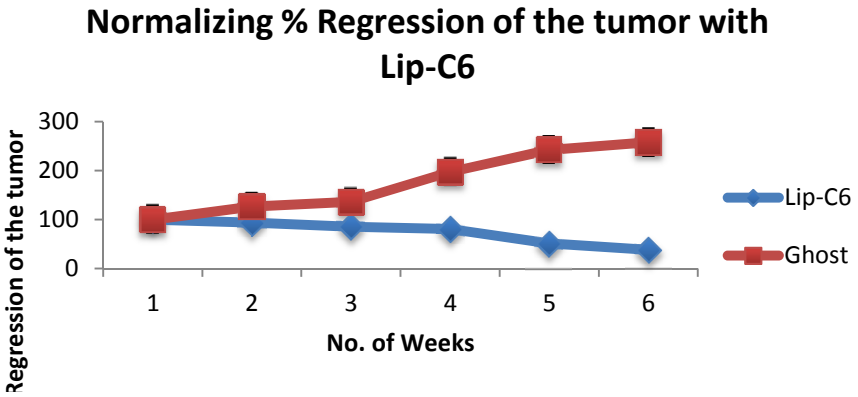
Nanoliposomal C6-ceramide prevents *in vivo* growth of SK-HEP-1 xenografted tumors in nude mice Model 1

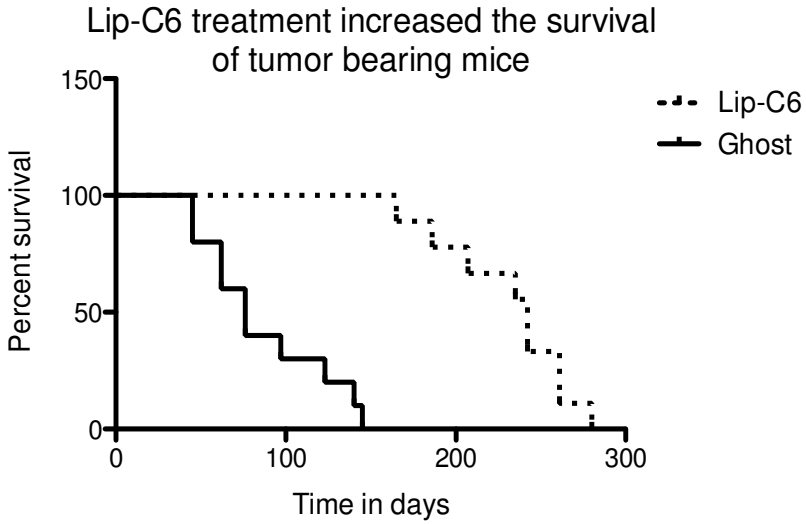


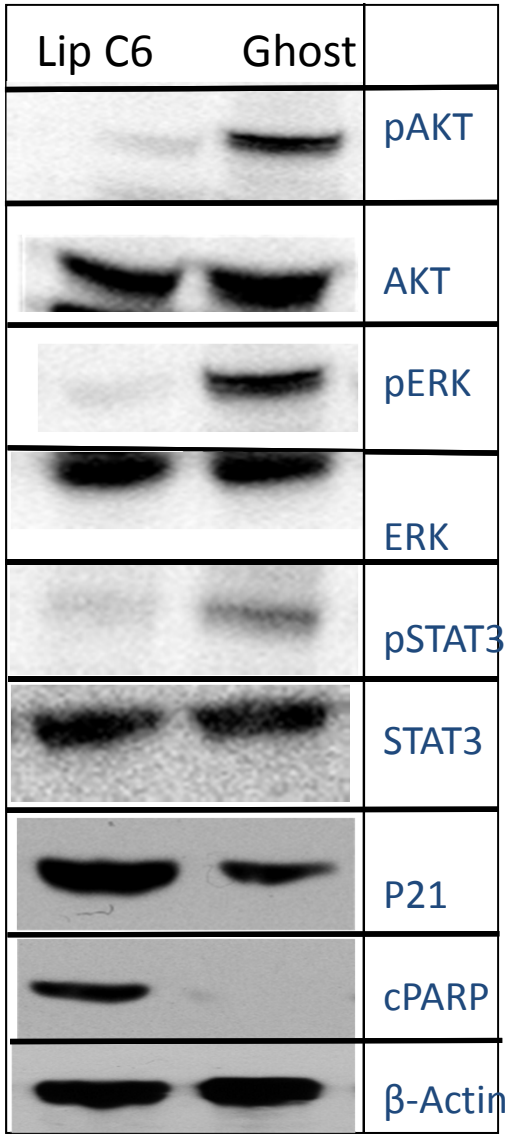
In Vivo Immunohistochemistry



CNL Regresses immune-tolerant intrasplenic HCC model







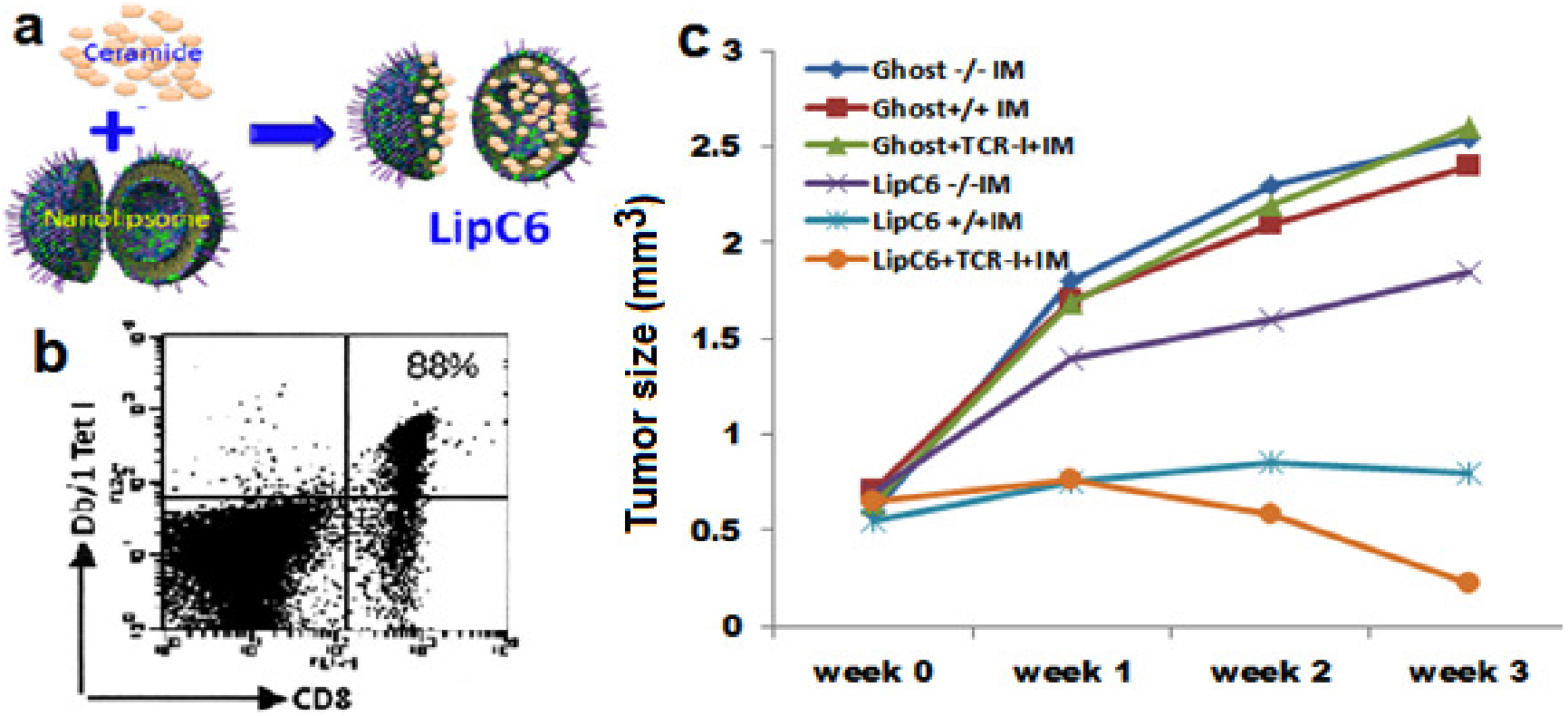
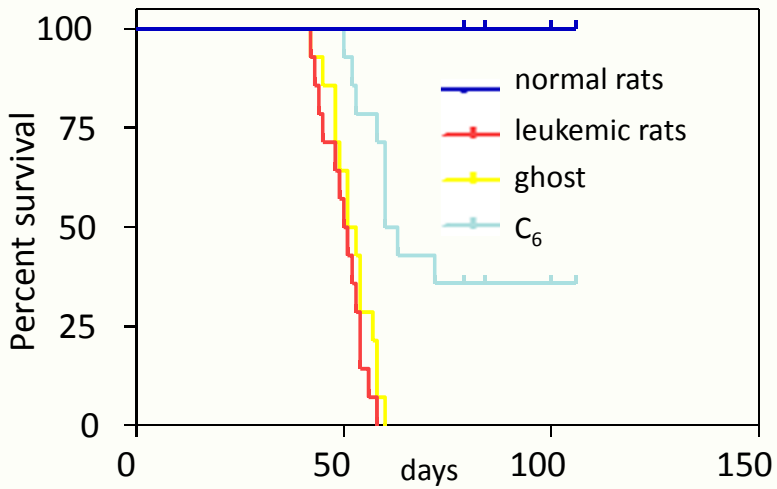


Figure 3. The anti-tumor/immunomodulatory effect of LipC6 in HCC. a. Nanotechnology is used to make nanoliposome-loaded ceramide (LipC6). b. The frequency of tet-I^{+}CD8^{+} T cells (TCR-I) in CD8 T-cell receptor for T antigen epitope-I transgenic mouse. Lymphocytes from lymph nodes and spleen in 416 mice are stained with fluorochrome-conjugated CD8 and tetramer-I and then used to conduct the flow cytometry assay for determining the frequency of TCR-I in CD8 T cell. c. Combination of LipC6 treatment and TCR-I T-cell following by immunization with Tag-transformed B6/WT-19 cells shrink the established tumors in our clinically-relevant murine model. Tumor-bearing mice with the comparable tumor volume are received the indicated treatment. Weekly Magnetic Resonance Imaging (MRI) is conducted to monitor tumor growth. The represent results in one experiment are shown.

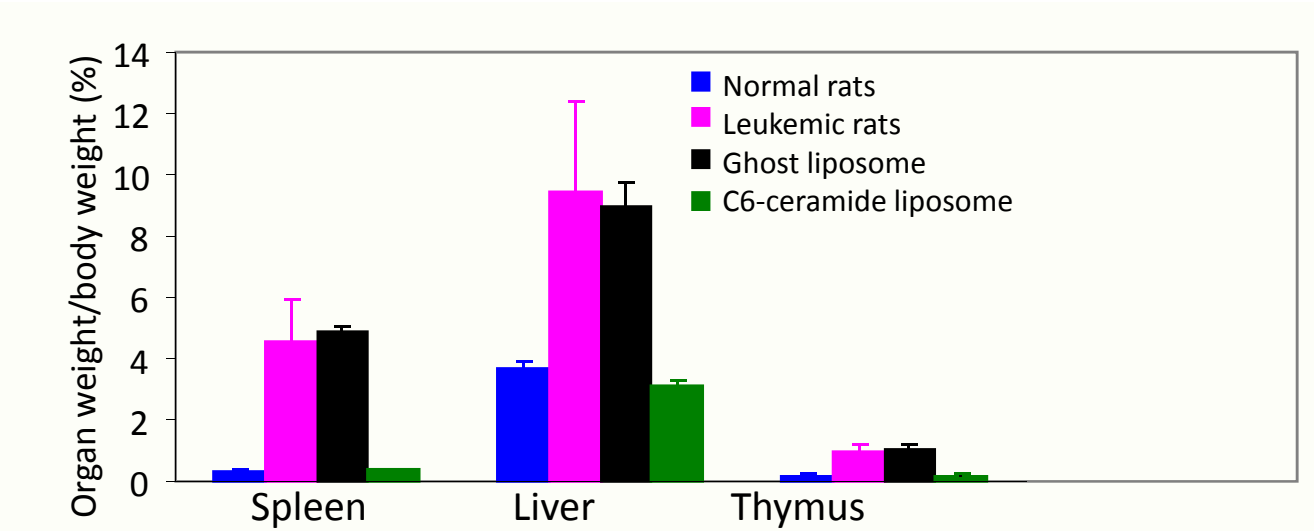
CNL Treatment Induces Remission in LGL Leukemic Rats

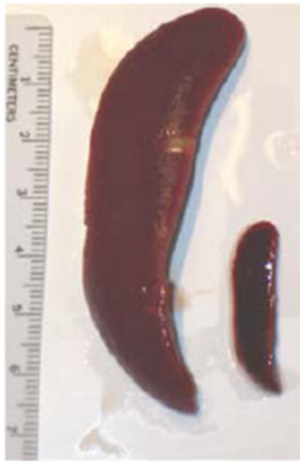


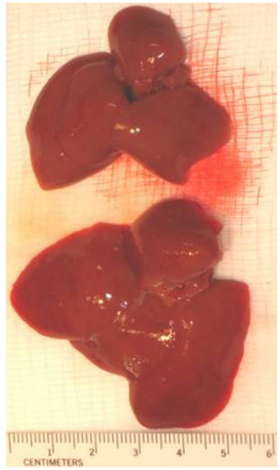
Kaplan–Meier survival curves

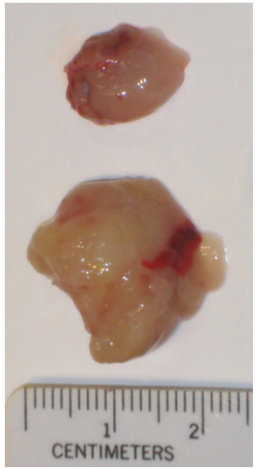
N = 14

The CNL reduced weights of organs infiltrated with leukemic cells

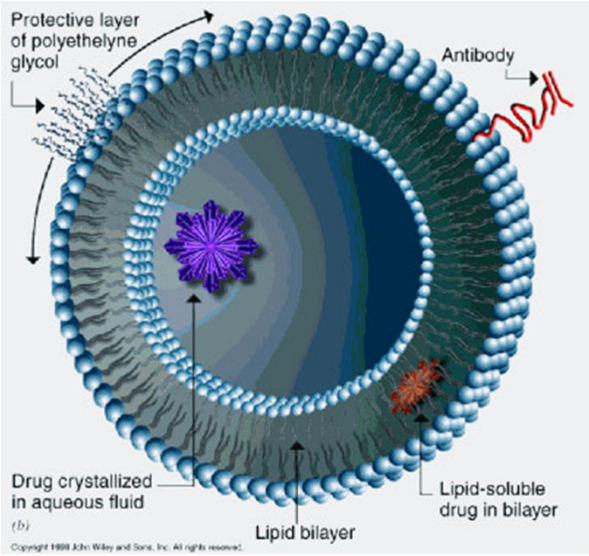








Second Generation Products
Combinatorial CNL Therapeutics



Ceramide + gemcitibine

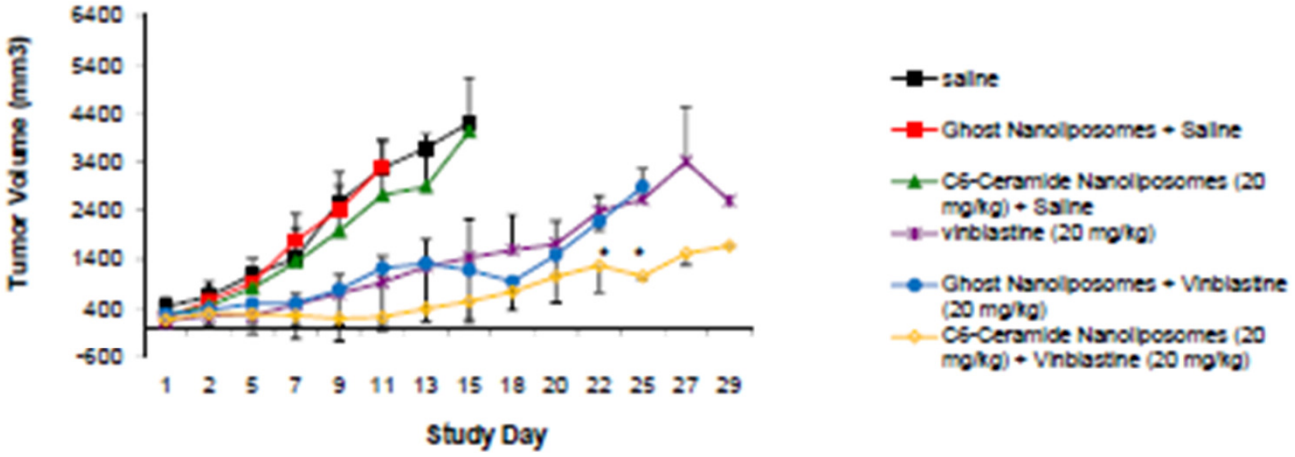
Ceramide + vinblastine

Ceramide + sorafinib

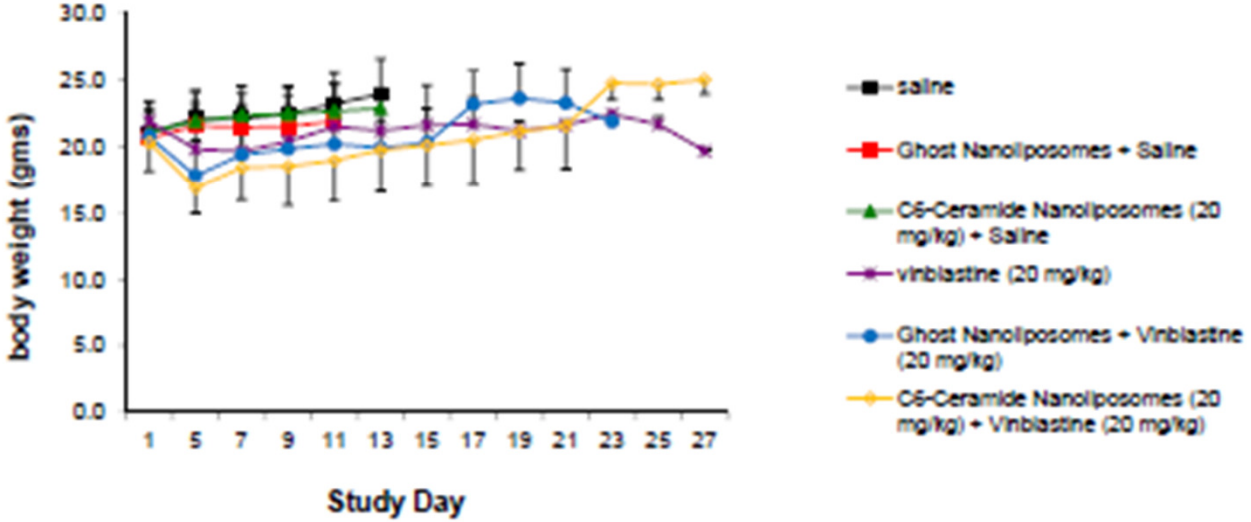
Ceramide + tamoxifen

In vivo efficacy of Combinatorial CNL
– In a LST174T xenograft Model –

5A



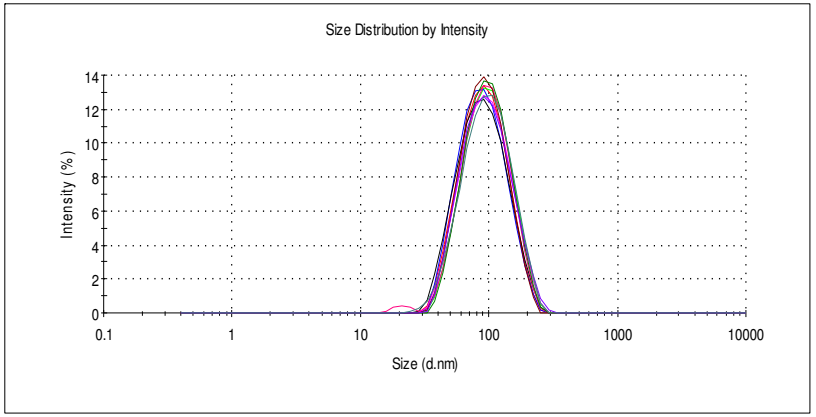
5B



Scale Up

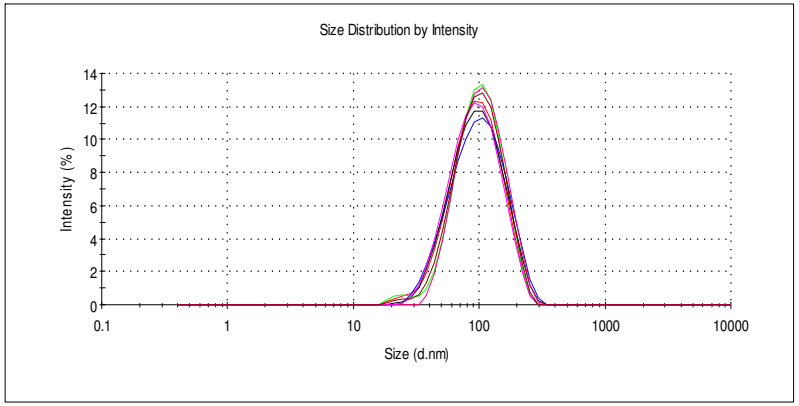
Experiment ID	Diameter (nm)	ZP (mV)
JB2-113-1	82	-10.8
JB2-113-2	87	-10.6
JB2-113-3	81	-11.4
JB2-113-4	80	-11.4
JB2-113-5	86	-11.4
JB2-113-6	82	-11.4
JB2-113-7	83	-11.2
JB2-113-8	87	-11.7
JB2-113-9	88	-11.7
JB2-113-10	87	-11.3

Average	84	-11.3
Standard Deviation	3	0.4



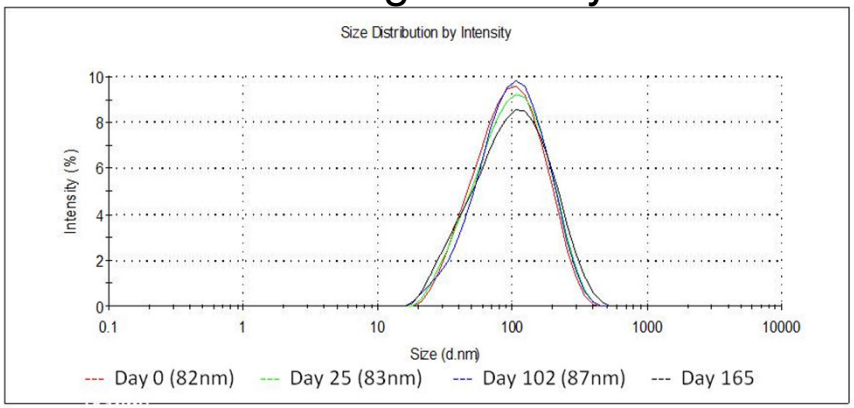
Experiment ID	Diameter (nm)	ZP (mV)
JB2-114-1	84	-10.8
JB2-114-2	88	-10.7
JB2-114-3	86	-11.6
JB2-114-4	87	-11.3
JB2-114-5	92	-10.9
JB2-116-1	83	-11.0
JB2-116-2	88	-11.1

Average	87	-11.0
Standard Deviation	3	0.3

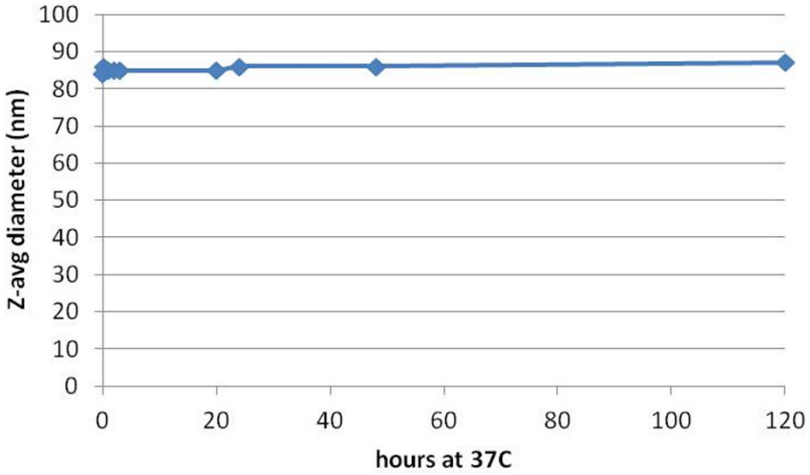


Stability

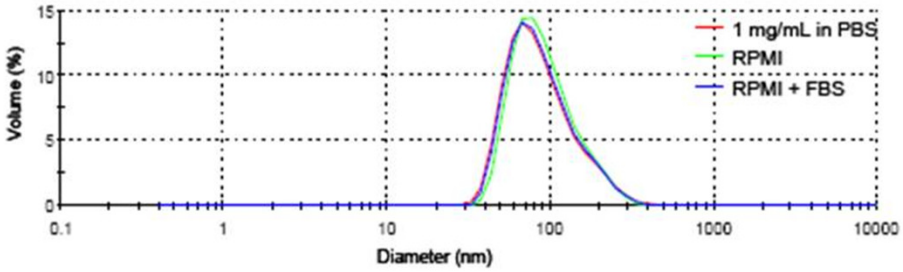
Storage stability



Stability @ 37C



Stability with serum (10%)



Nanotechnology Characterization Laboratory
National Cancer Institute
www.ncl.nci.gov

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Planned Clinical Trial

- **Primary End Point**
  - To define maximum tolerate dose (MTD) and dose limiting toxicities (DLT)
  - To establish the PK in humans
- **Secondary End Point**
  - To evaluate the safety, tolerability, and adverse event profile of ceramide nano liposome
  - To describe the response rate, time to disease progression and response duration
- **Study design and treatment plan**

  Toxicology data demonstrated that ceramide nanoliposome is safe in mice at therapeutic dose (36mg/kg in mice = 2.93mg/kg in human). Additionally, no obvious toxicities were observed at twice efficacious doses. Hence, current estimate are to start at 0.3mg/kg in human study.
- **Drug Administration**

  CNL will be administered intravenously at indicated doses twice weekly (Monday and Thursday). The medication will be infused over 1 hour period of time. To prevent infusion reaction, pre-medications will be administered 30 minutes prior to infusion. The pre-medications include diphenhyramine 25mg P.O; odansetron 16mg P.O. and dexamethasone 12mg P.O.

  Dose Levels / Cohorts

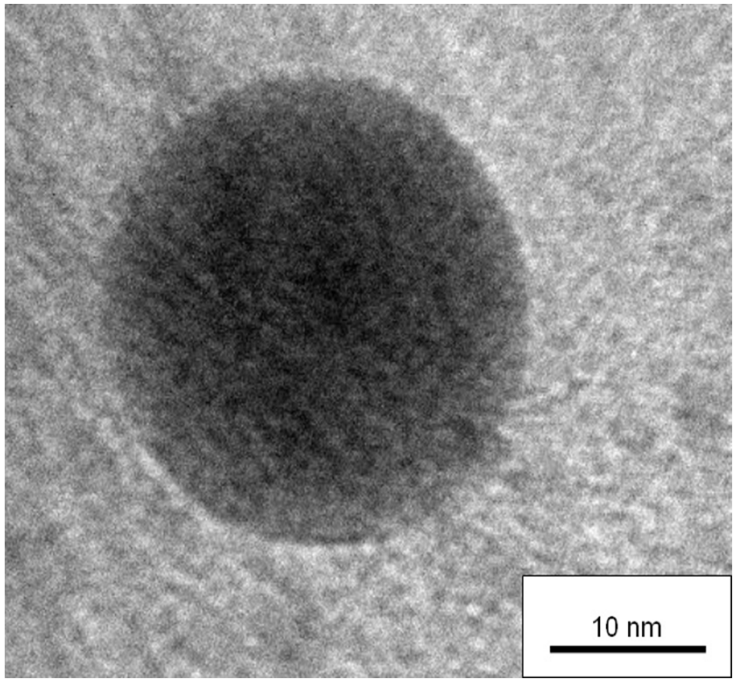
  This is a single arm, open label phase I dose escalation study with a cohort expansion. A classic "3+3" design will be used the study.

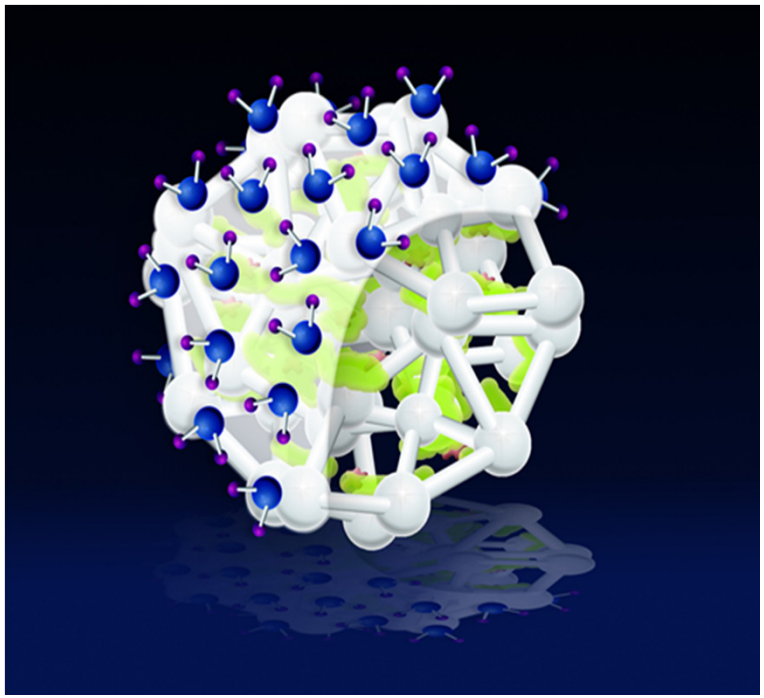
Dose levels	Ceramide Nanoliposome (mg/kg) twice per week
-I	0.3mg/kg
I	1mg/kg
II	2 mg/kg
III	2.5 mg/kg
IV	3mg/kg
V	3.5mg/kg
VI	4mg/kg

Conclusion

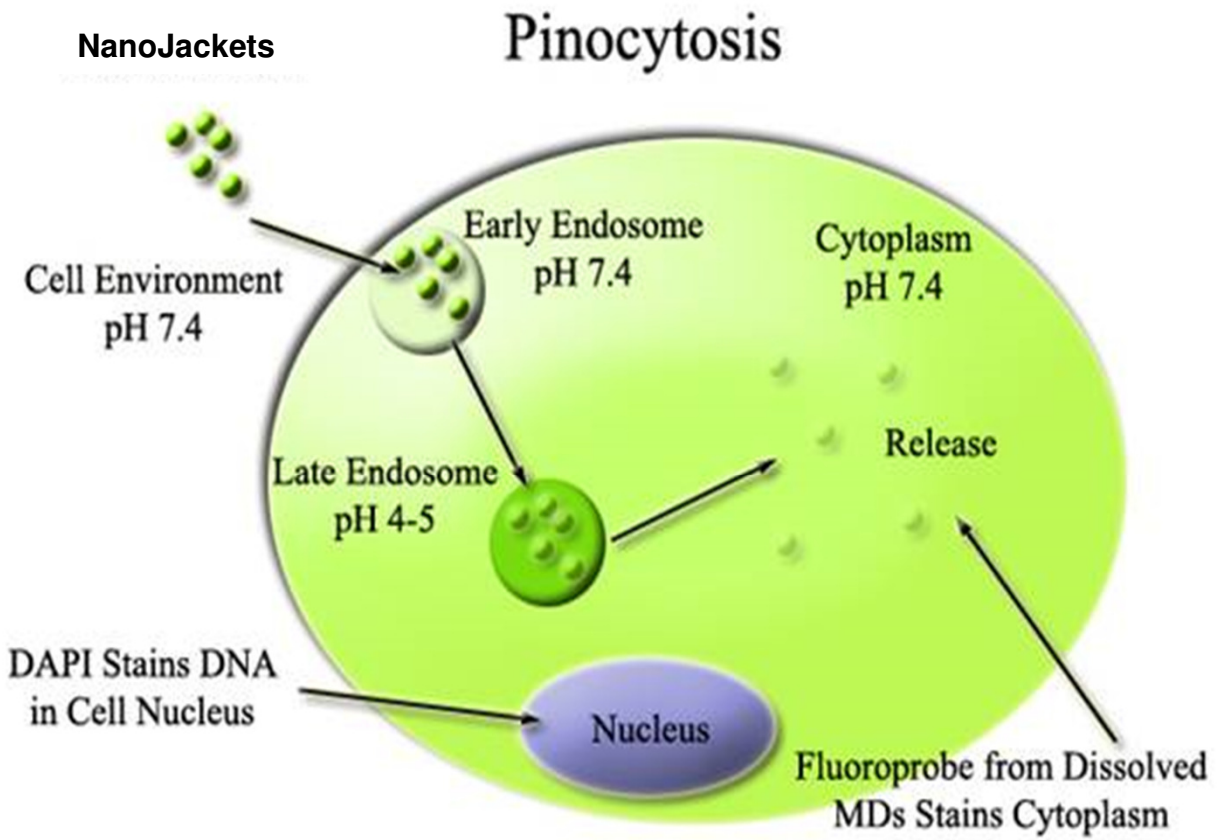
- **Nanotechnology has the potential to “deliver” the promise of ceramide-based pharmaceutics**

Calcium Phosphosilicate NanoParticles
NanoJackets

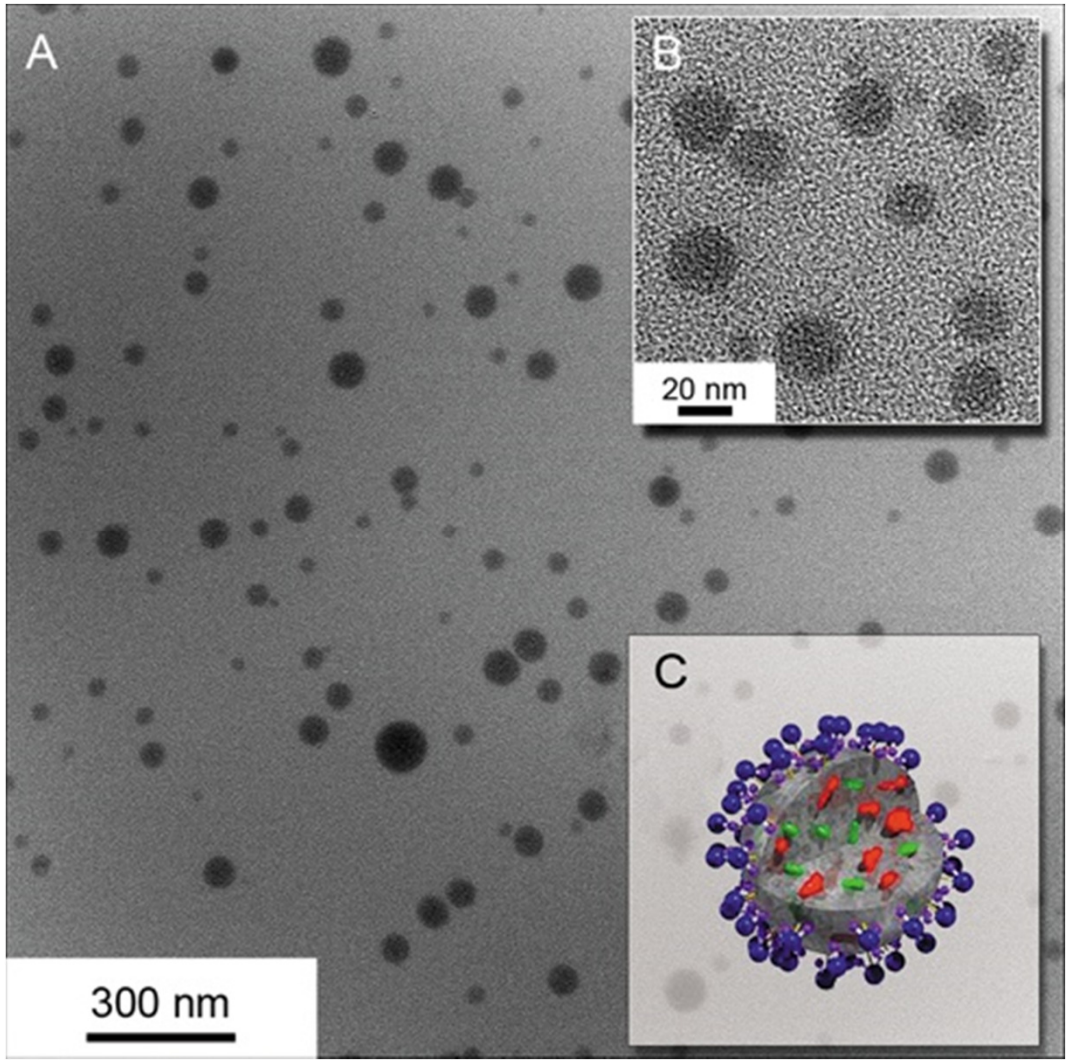




NanoJackets are Molecular Smart Bombs;
Encapsulated components are released as a function of pH

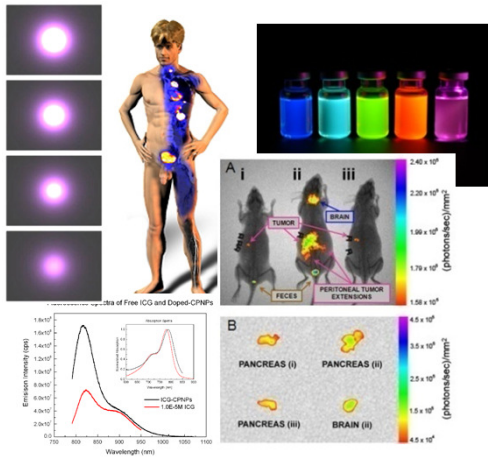


TEM of ICG-loaded CPSNP

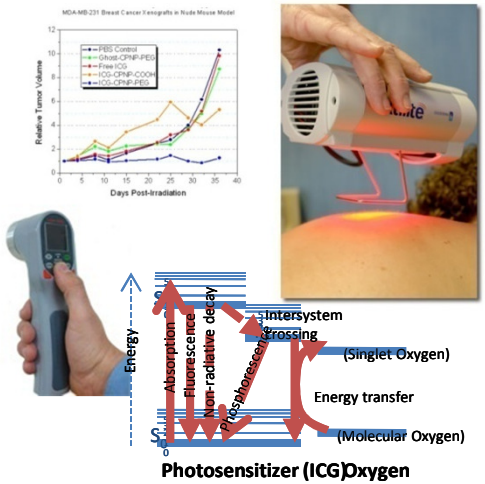


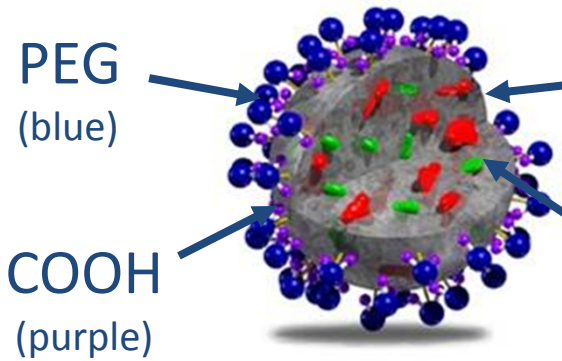
Calcium Phosphosilicate Nanoparticle (CPSNP) as Theranostics

Near Infrared Imaging



Minimally Invasive Therapy





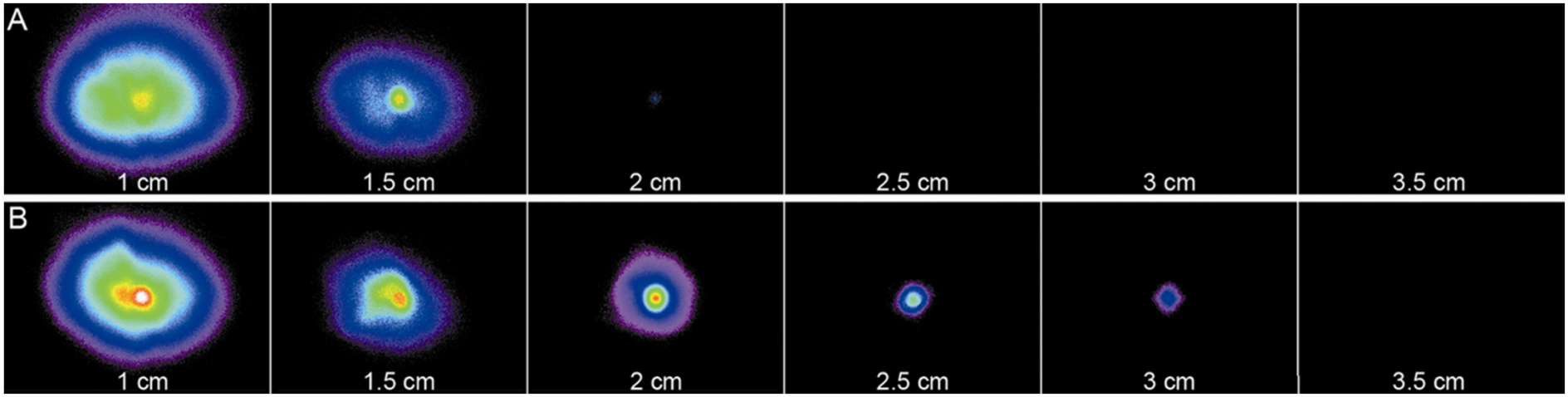
PEG
(blue)

CPSNP
(gray)

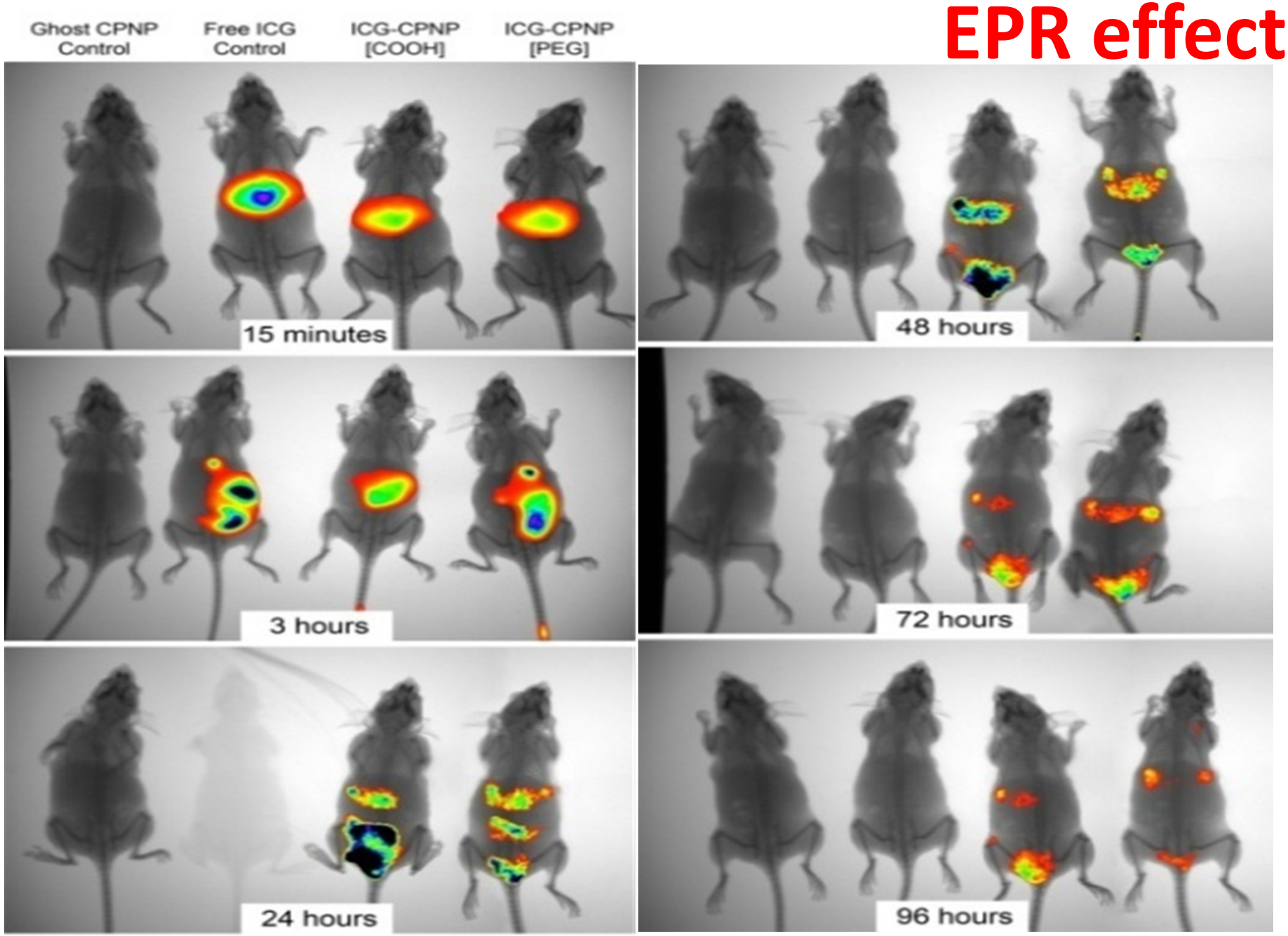
COOH
(purple)

Indocyanine Green
(red/green)

Comparative Fluorescence Signal Intensity as Function of Depth in Porcine Muscle Tissue

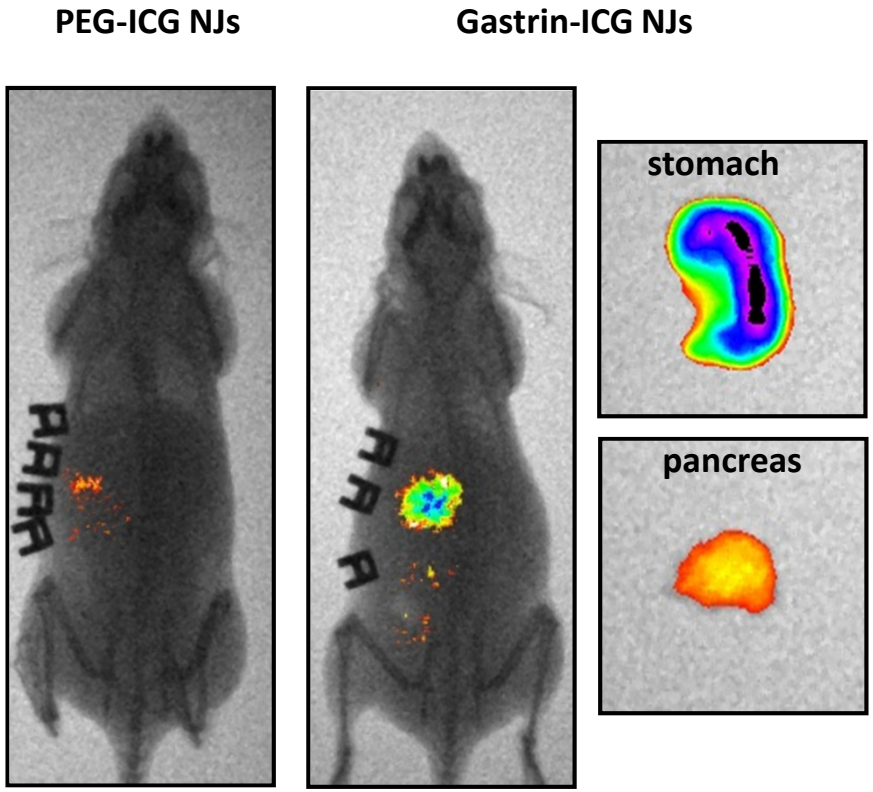


EPR effect



Altinoglu et al ACS Nano 2008

CPSNP Targeting – Pancreatic Cancer

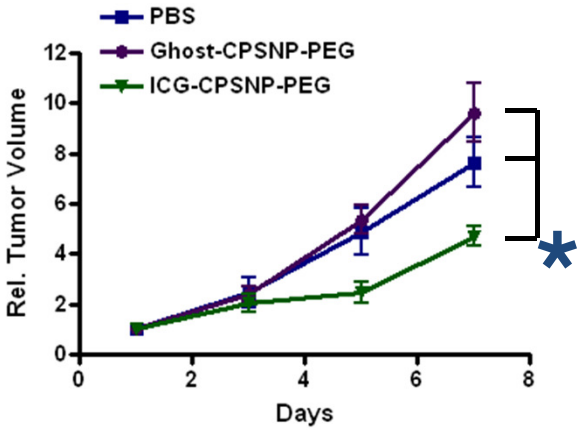


• Attachment of a gastrin peptide fragment to the NJ results in specific localization of ICG NanoJackets to the stomach and pancreas – sites of gastrin receptor expression, 24 hours post-systemic tail vein injection

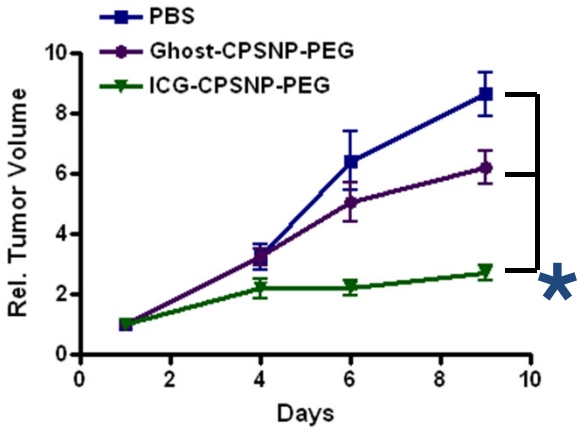
B.M. Barth et al., ACS Nano, 2010.

PhotoImmuno Nanotherapy (PINT) Exerts Robust In Vivo Efficacy

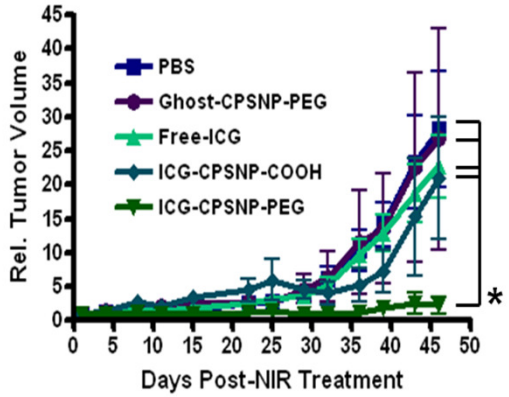
410.4 Breast Cancer (Balb/cJ Mice)



410.4 Breast Cancer (NOD-SCID Mice)

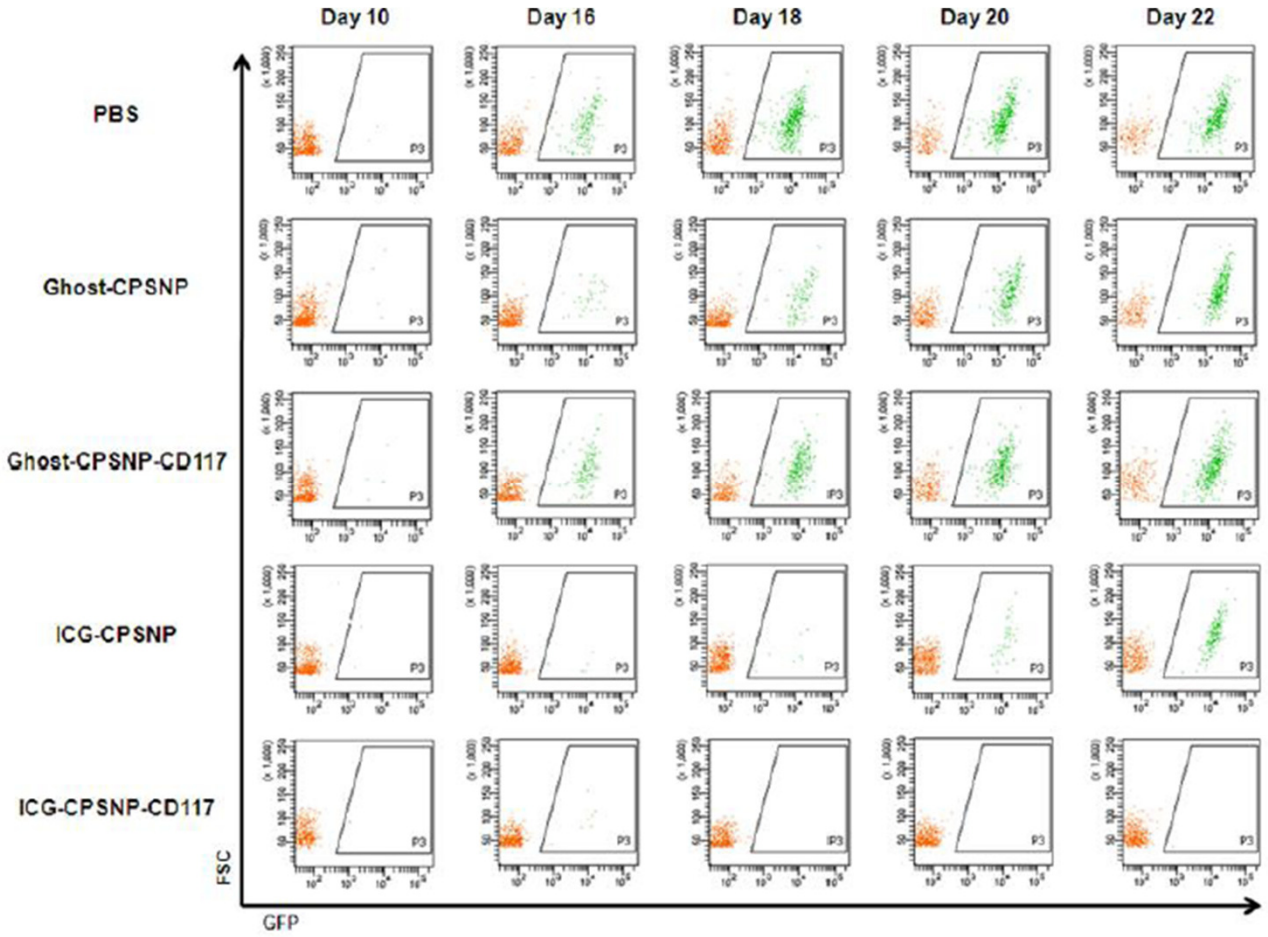


MDA-MB-231 Breast Cancer (Athymic Nude Mice))



Immuno-targeted ICG-CPSNP Photodynamic Therapy of Chronic Myeloid Leukemia

(murine 32D-P210-GFP chronic myeloid leukemia in C3H/HeJ mice)



Conclusion

- Nanotechnology has the potential to “deliver” the promise of light-based pharmaceutics

Nanotechnology Enables Personalized Medicine

The future of medicine will be defined by designer drugs applied to individuals, tailored to a specific molecular or metabolic pathology.

This is the essence of personalized medicine.

Very Few Drugs that Target Mutated Proteins

Imatinib (Gleevec), Nilotinib, Desatinib	
Bcr-Abl	CML
Vemerafenib (Zelboraf)	
Braf V600E	melanoma
Crizotinib	
translocation of ALK gene	non small cell lung
Afatinib	
T790M	EGFR mutants
Ivacaftor (Kalydeco)	
CFTR G551D	Cystic Fibrosis

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CFTR G551D	Cystic Fibrosis

Yet, Resistance develops due to secondary mutations

Specificity and Selectivity of siRNA-encapsulated Nanoparticles

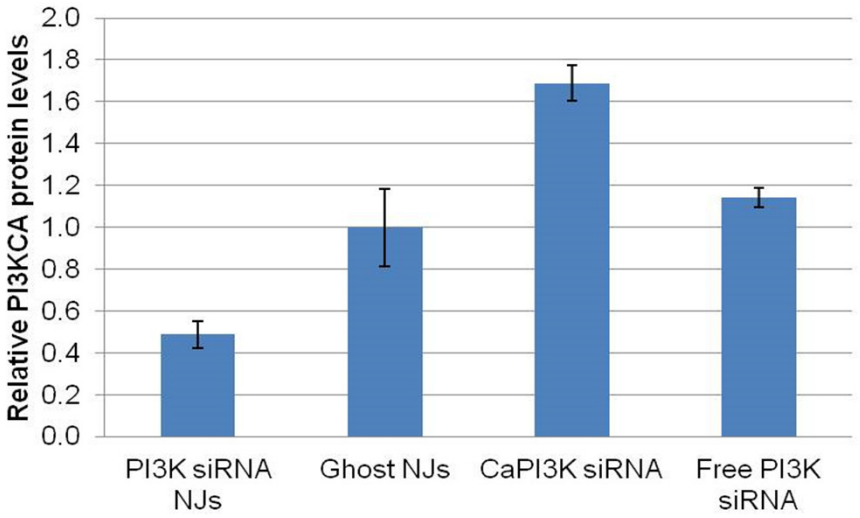


Figure 3. In vitro knockdown of PI3KCA protein levels following PI3K 3140A>G siRNA NanoJacket treatment. T47D human breast cancer cells were treated with free siRNA, siRNA pre-associated with calcium, ghost NanoJackets (NJs) containing no siRNA or NanoJackets containing siRNA for 48 hours. PI3KCA protein levels were measured by western blot and normalized to B-actin controls. Average protein levels of 3 replicate cell treatments are shown with standard deviations.

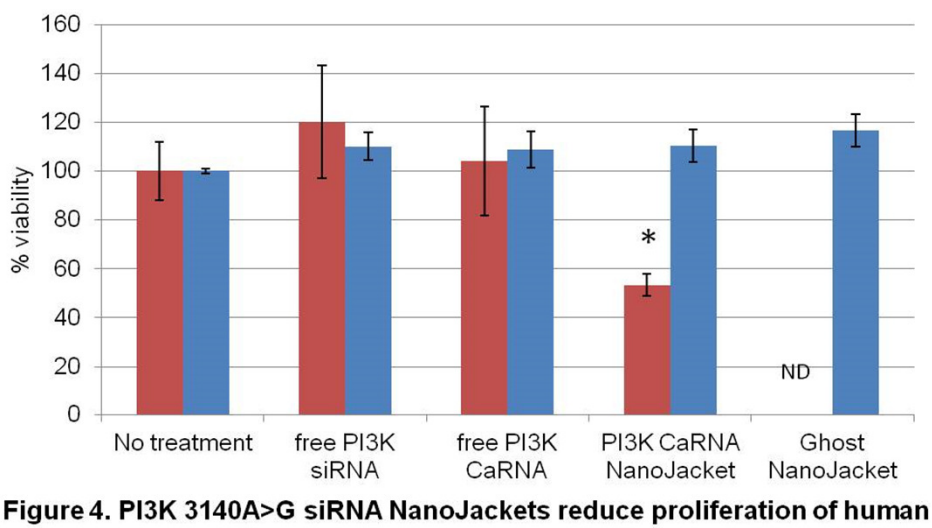
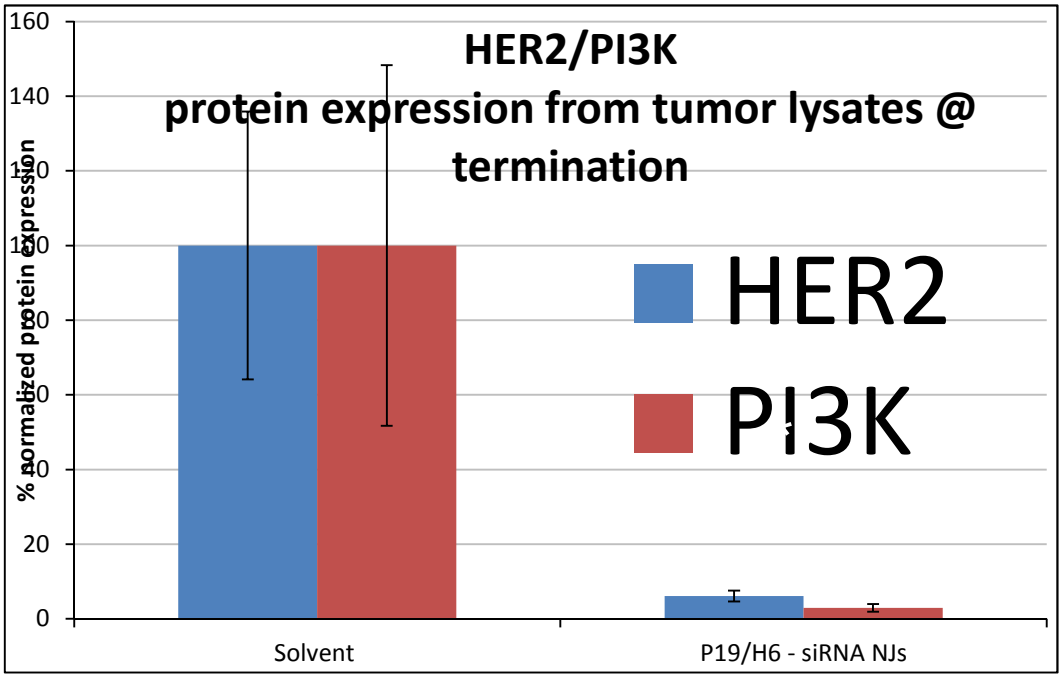


Figure 4. PI3K 3140A>G siRNA NanoJackets reduce proliferation of human breast adenocarcinoma cells that contain the targeted mutation but do not affect those without the targeted. MDA-MB-453 cells (red bars), which contain the 3140 A>G PI3K mutation or MDA-MB-231 cells (blue bars), which do not contain the targeted mutation, were treated with free siRNA, free siRNA bound to calcium (CaRNA), PI3K CaRNA NanoJackets or ghost NanoJackets (without siRNA) for 48 hours and proliferation was measured by a MTS non-radioactive proliferation assay. The average +/- standard error for each treatment is shown. * indicates $p<0.05$ using a t-test analysis in comparison to untreated control for that cell line. ND = not done.

In Vivo Knockdown of Target Proteins

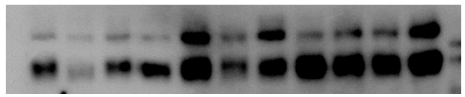


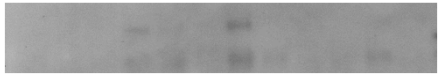
Normalized Expression		
Treatment	**PI3K**	**HER2**
Solvent	100 +/- 48.3%	100 +/- 35.9%
P19/H6 siRNA NJs	**2.9 +/- 1.0%**	**6.1 +/- 1.4%**

Solvent (5% Dextrose)

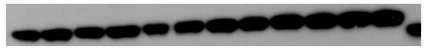
P19/H6 siRNA NanoJackets

HER2 MW 185
PI3K MW 1





actin MW 45



Targeting Her2/PI3K with siRNA NJ in vivo

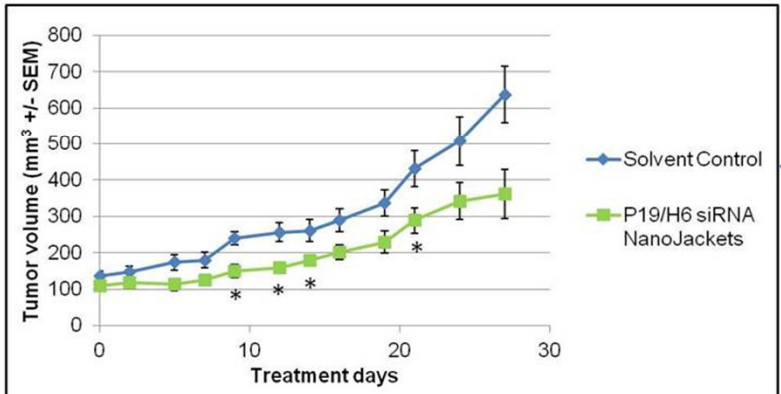
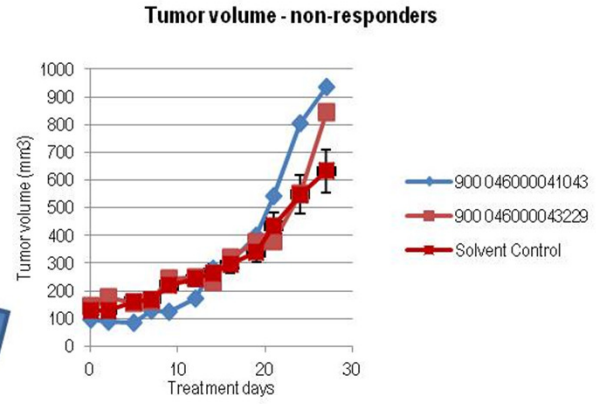
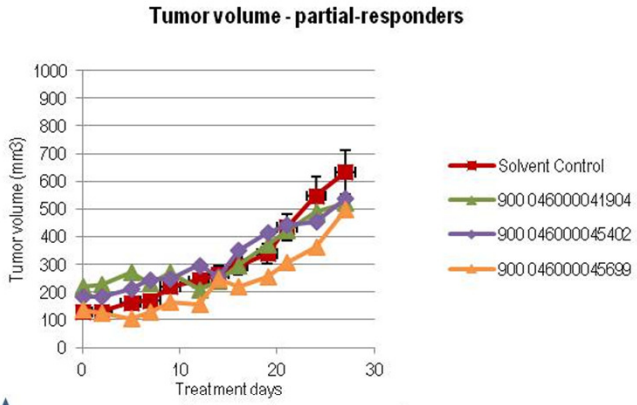
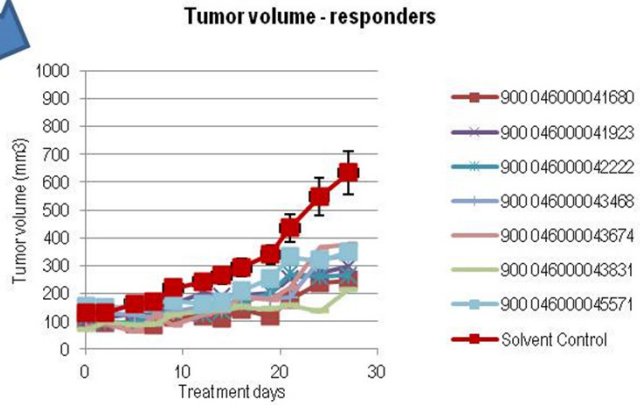


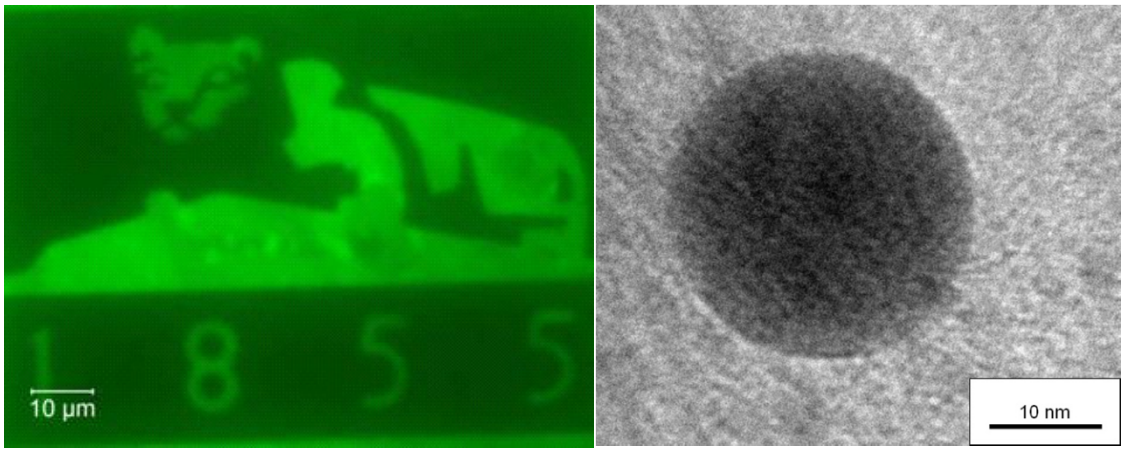
Figure 4. Efficacy of the initial P19/H6 siRNA NanoJacket prototype. A) Orthotopic HCC1954, human breast cancer tumors, were established in SCID-CB17 mice and treated three times weekly with i.v. administrations of solvent (5% dextrose) or P19/H6 siRNA NanoJackets (2.5mg/kg). Tumor volume is shown as the median tumor volume +/- standard error. * = p<0.05



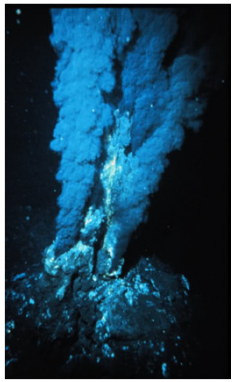




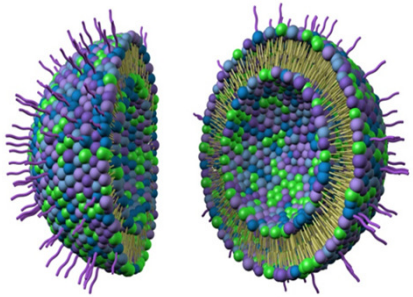
Nanotechnology holds the promise to enable Personalized Medicine



The ORAL Platform
Oral Resistant Archaeal Liposomes

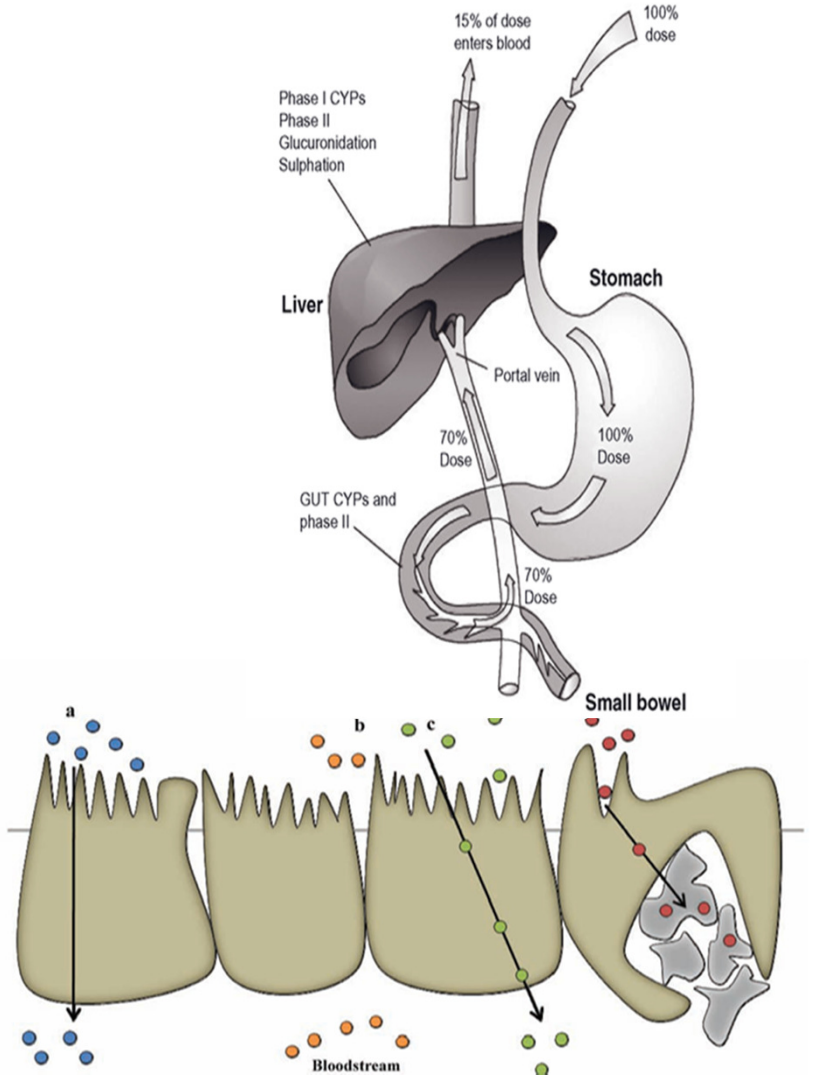


- **Archaebacteria - resistant to pH and enzymatic 'digestion'**
- **Manufacturability**
  - **Synthetic archaeal lipids commercially available**
  - **Monodispersed & homogeneou even at low pH**
- **Efficient & flexible protein loading**
- **Patent protection**
- **Differentiated from competition**
  - **Protection and delivery in one platform**



Oral Delivery of Biologics

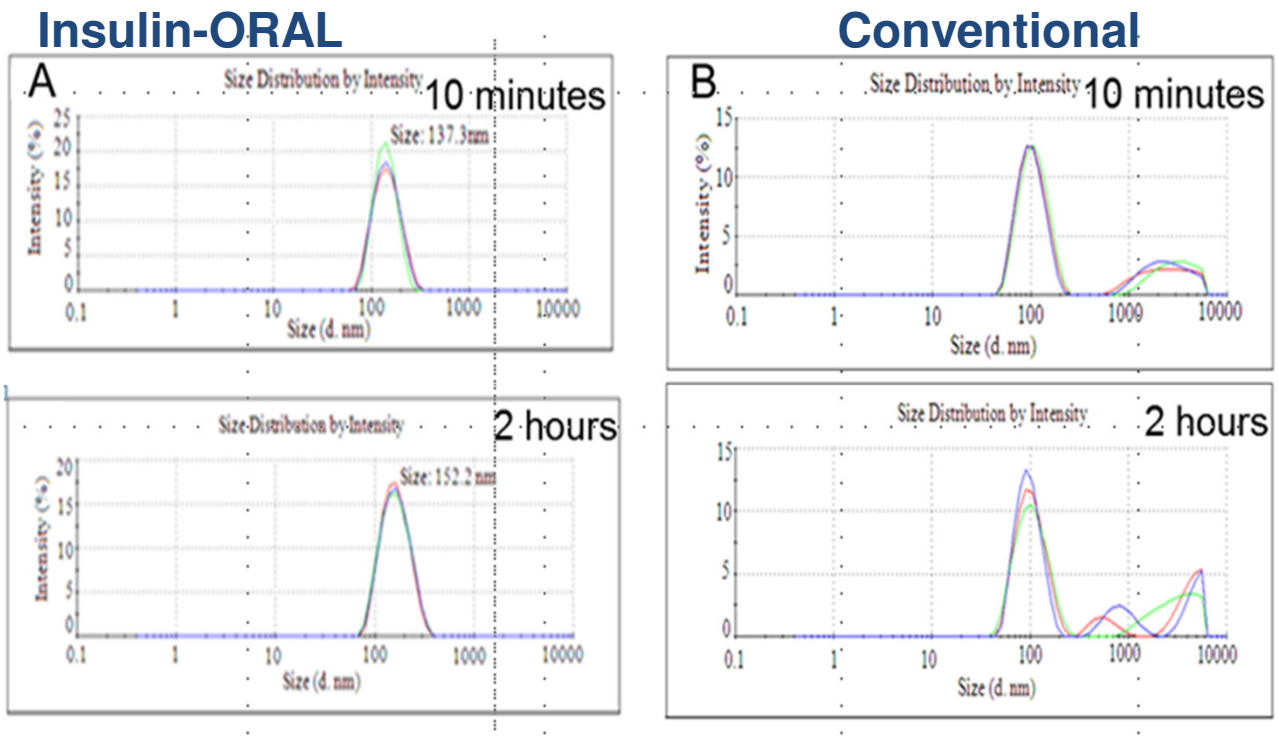
- "Holy grail" of drug delivery
  - high hassle-factor of i.v. or subQ
  - line extensions
- Barriers to peptide/protein absorption through the gut
  - "Digestion" = low pH + enzymes
  - Transepithelial delivery to systemic circulation



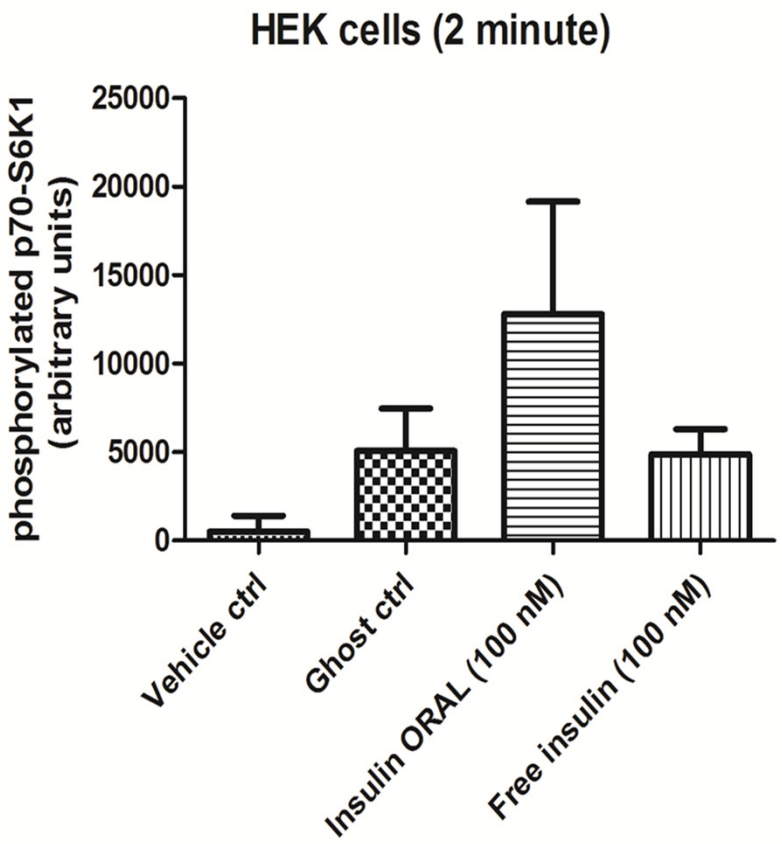
oraceuTX LLC

ORAL Characterization

pH 1



Insulin-Oral Delivery

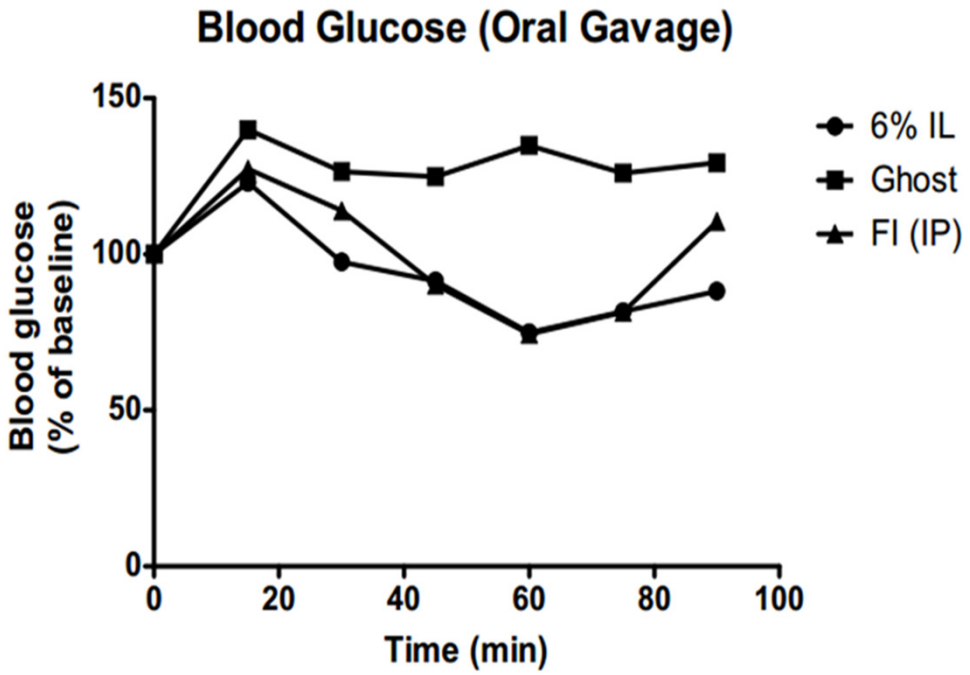


Insulin-ORAL is bioactive

Insulin-ORAL displays greater potency than free insulin

Bioactive in adipose and muscle cell lines

Insulin-ORAL – Protection and Delivery



Normal rodents

Insulin-ORAL delivered orally comparable to injected insulin

- **Nanotechnology holds the promise to enable Oral Delivery of Biologics**

The University of Virginia

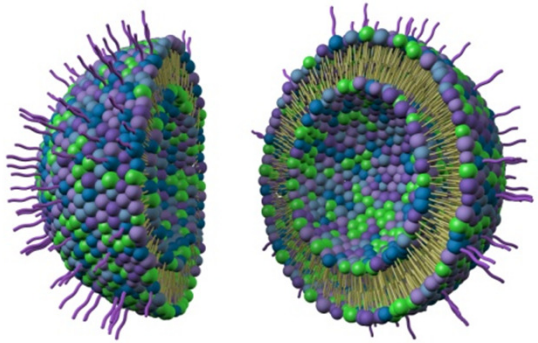
Todd Fox
Brian Barth
Tom Stover
Sriram Shamnugavelandyu
James Kaiser
Nicole Divittore
Nicole Keasy
Lindsey Ryland
Daniza Crespi-Gonzalez
Megan Young
Jeremy Haakenson

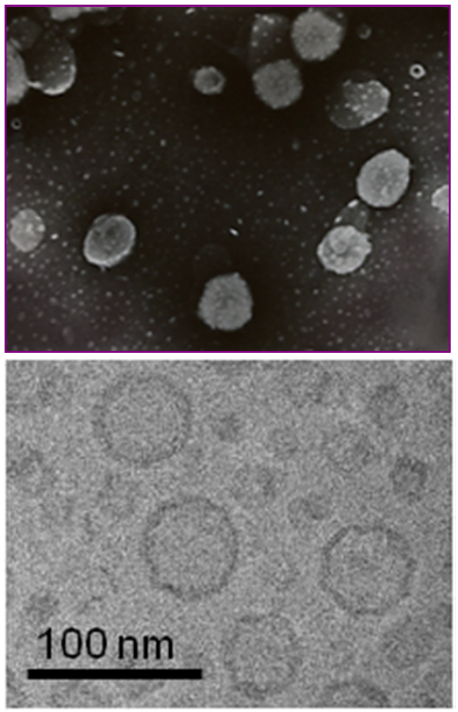
Kevin Staveley-O'Carroll
Hezipah Tagaram
Guongfu Li
Yixing Jiang
Tom Loughran
Xin Liu
Jill Smith
David Claxton
HG Wang
Jong Yun

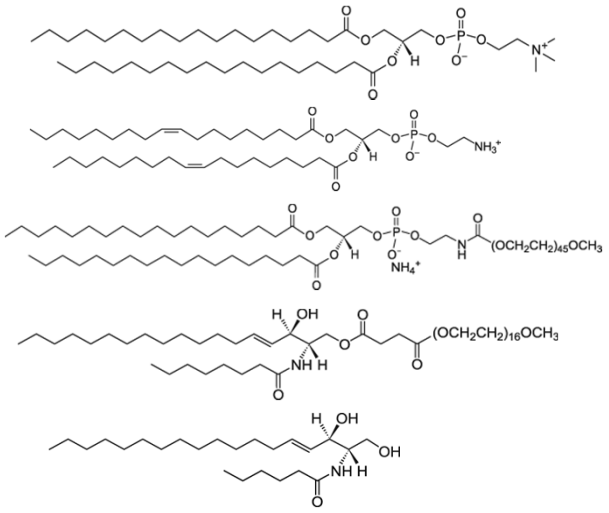
James Adair
Sarah Rouse
Erhan Altinoglu
Tom Morgan
Peter Butler
Peter Eklund
Nadine Barrie-Smith
Victor Ruiz-Velasco

The Ceramide NanoLiposome (CNL)

- Composed of a mixture of DSPC:DOPE:DSPE-PEG:C8Ceramide-PEG750:C6 Ceramide in a 3.75:1.75:0.75:0.75:3 molar ratio
- Total lipid concentration of 25 mg/mL, C_6 Ceramide concentration of 3.51mg/mL
- Incorporates C_6 Ceramide into the lipid bilayer resulting in aqueous solubility that allows systemic administration (iv), enhanced circulation time, increased cellular uptake and protection from degradation
- DMFs available for all but C_8 Ceramide-PEG750







CMC overview

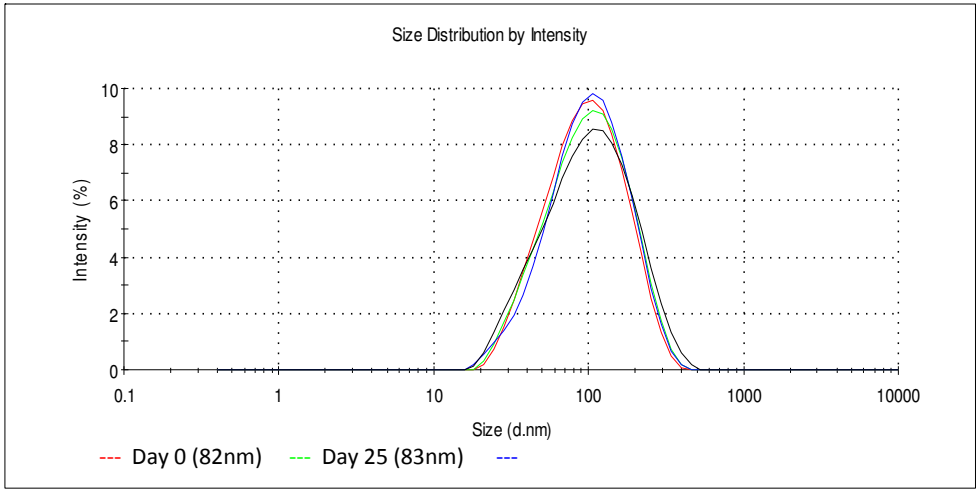
Component materials:

- 1,2-Distearoyl-*sn*-Glycero-3-Phosphocholine (DSPC)
- 1,2-Dioleoyl-*sn*-Glycero-3- Phosphoethanolamine (DOPE)
- PEG(2000)- 1,2-Distearoyl-*sn*-Glycero-3-Phosphoethanolamine (DSPE-PEG)
- N-Octanoyl-Sphingosine-1-[Succinyl(Methoxy(Polyethylene Glycol) 750 (C8CeramidePEG750)
- N-Hexanoyl-D-erythro-Sphingosine (C6 Ceramide)

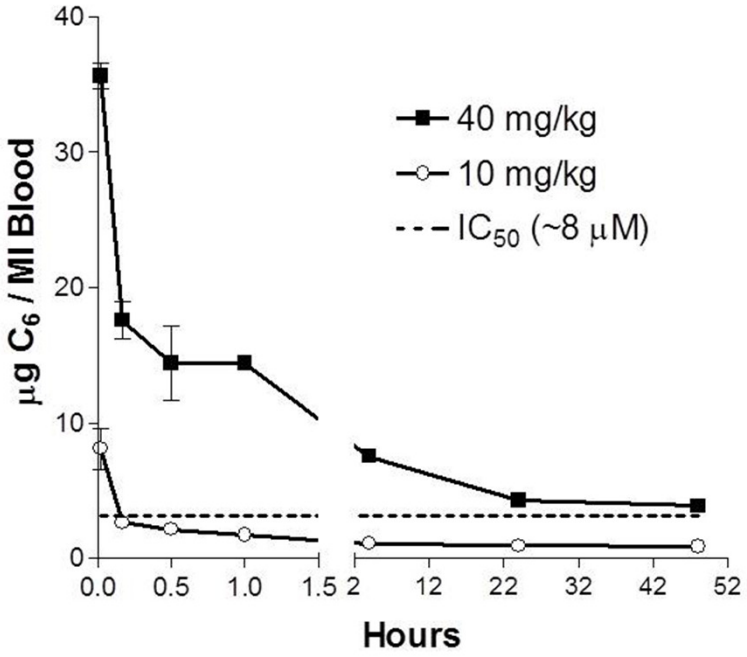
DMF/GMP availability: All components except C8CeramidePEG750 have a DMF and are available in GMP grade.

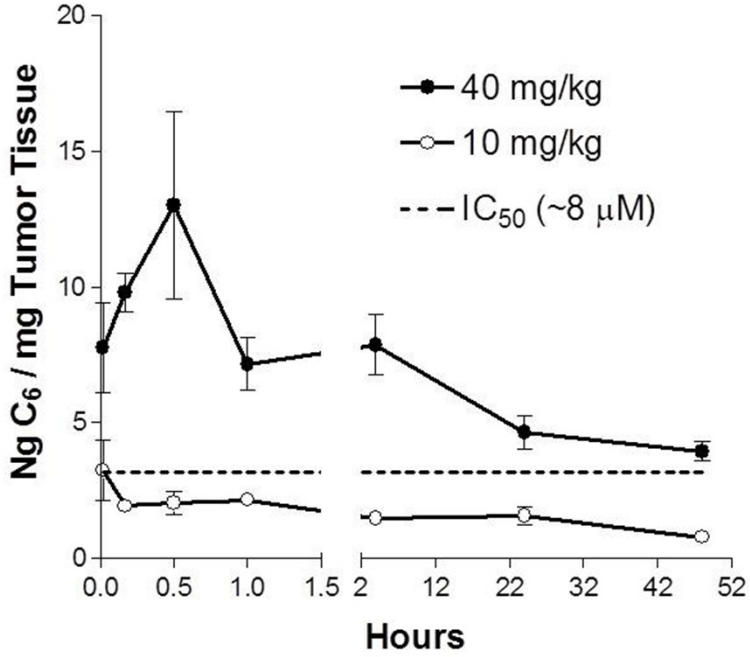
Manufacture: Pressurized homogenization formulated in sterile phosphate buffered saline at pH 7.4 with no additional excipients.

Batch ID	Diameter (nm)	ZP (mV)
JB2-114-1	84	-10.8
JB2-114-2	88	-10.7
JB2-114-3	86	-11.6
JB2-114-4	87	-11.3
JB2-114-5	92	-10.9
JB2-116-1	83	-11.0
JB2-116-2	88	-11.1
Average	86.9	-11.1
Standard Deviation	3.0	0.3



Pharmacokinetics





Preclinical Testing by Penn State Hershey Medical Center

Ceramide Liposome (C_6)

$Time_{Max}$	Min	0
$Conc_{Max}$	ng/mg	38.5826
$Time_{Final}$	Min	1440
$Conc_{Final}$	ng/mg	4.3
AUC	Min*ng/mg	9698.469
$t_{1/2}$	Min	**677.8695**
$Clearance_{OBS}$	mg/(min*ng/mg)	0.0029

T ½ is 11.2 hours

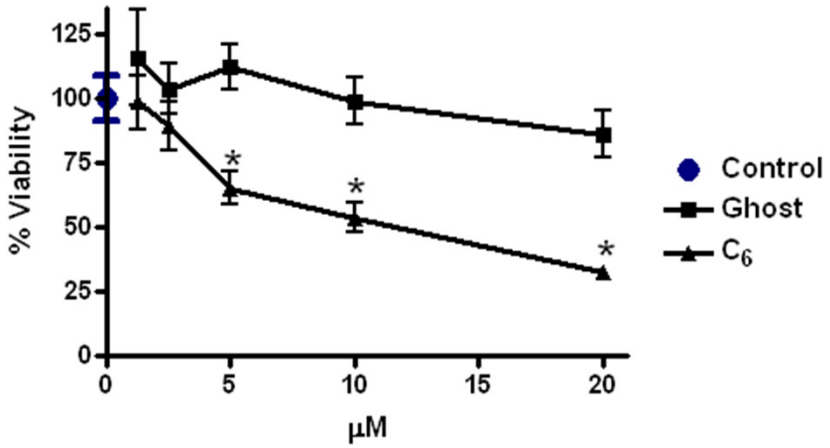
Pharmacokinetics

LC-MS detection of C_6 Ceramide
Sprague-Dawley rats

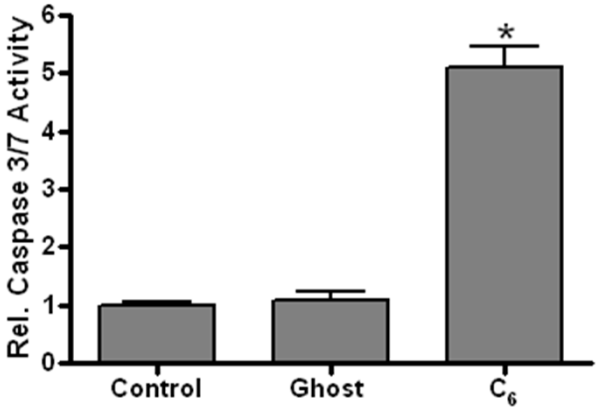
Day	Gender	Dose (mg/kg)	C_0 (µg/mL)	$AUC_{0\text{-}24h}$ (µg.h/mL)	AUC_{last} (µg.h/mL)	AUC_{inf} (µg.h/mL)	CL_{pred} (mL/min/kg)	Vss_{pred} (L/kg)	$T_{1/2}$ (h)	T_{last} (h)	C_{last} (µg/mL)
1	Male	9.00	19.52	22.52	22.52	28.53	5.26	4.53	12.14	24.00	0.35
		18.00	38.41	48.50	48.50	64.59	4.64	4.58	13.59	24.00	0.84
		35.00	156.10	104.96	104.96	145.99	4.00	4.39	16.72	24.00	1.71
	Female	9.00	18.95	20.90	20.90	27.71	5.41	5.28	13.50	24.00	0.36
		18.00	58.47	46.83	46.83	62.35	4.81	4.73	14.21	24.00	0.76
		35.00	159.25	101.91	101.91	135.96	4.29	4.23	14.23	24.00	1.67
4	Male	9.00	18.57	22.99	33.63	34.39	4.36	5.50	18.85	96.00	0.03
		18.00	54.22	56.72	87.68	89.97	3.33	4.56	19.08	96.00	0.08
		35.00	167.07	114.27	171.47	175.26	3.33	4.23	18.39	96.00	0.14
	Female	9.00	21.41	21.43	30.79	31.15	4.82	5.49	15.56	96.00	0.01
		18.00	64.75	50.14	71.82	72.58	4.13	4.55	15.11	96.00	0.03
		35.00	137.78	106.37	160.01	162.60	3.59	4.41	16.68	96.00	0.10

Nanoliposomal C6-ceramide decreases the viability of SK-HEP-1 cells *in vitro* by inducing apoptosis

A



B



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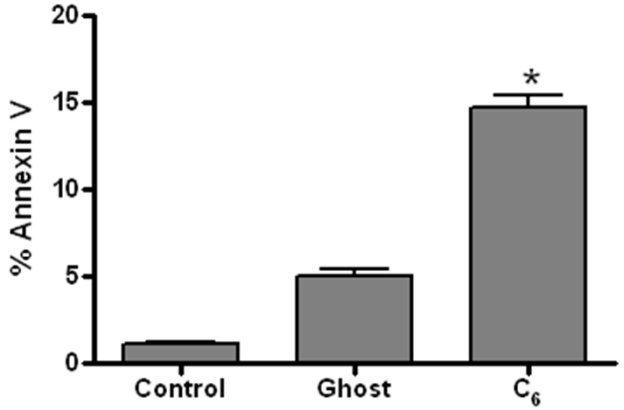
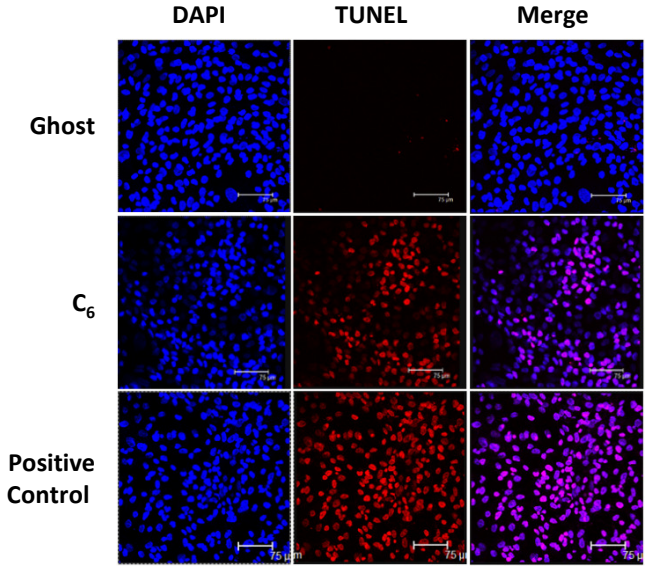
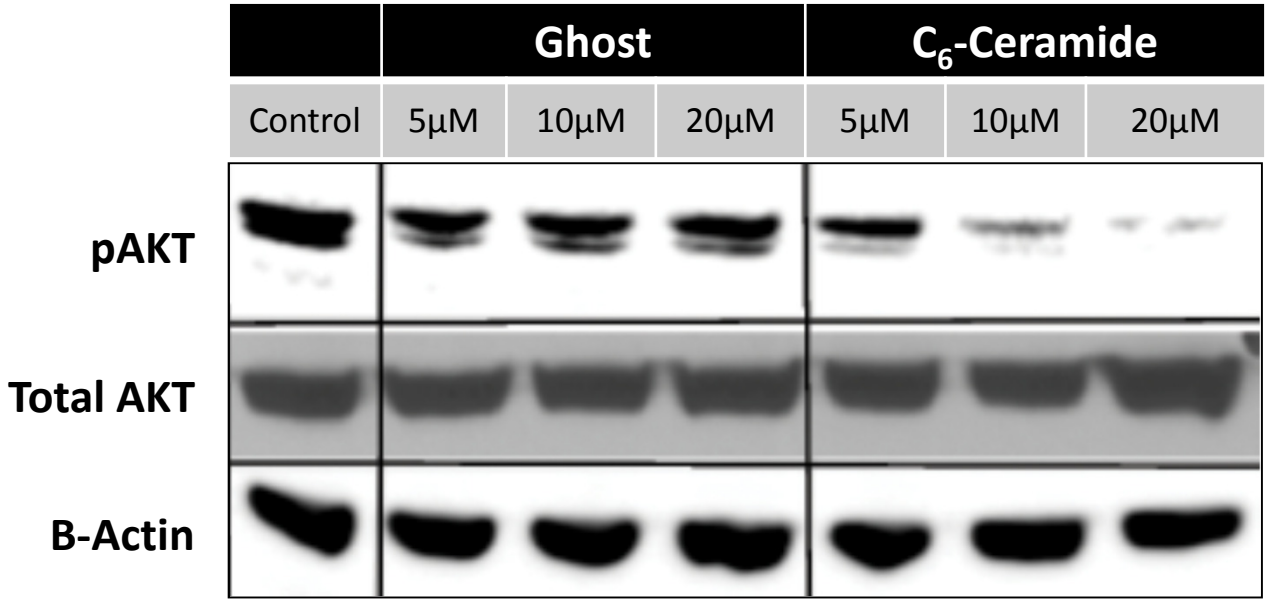
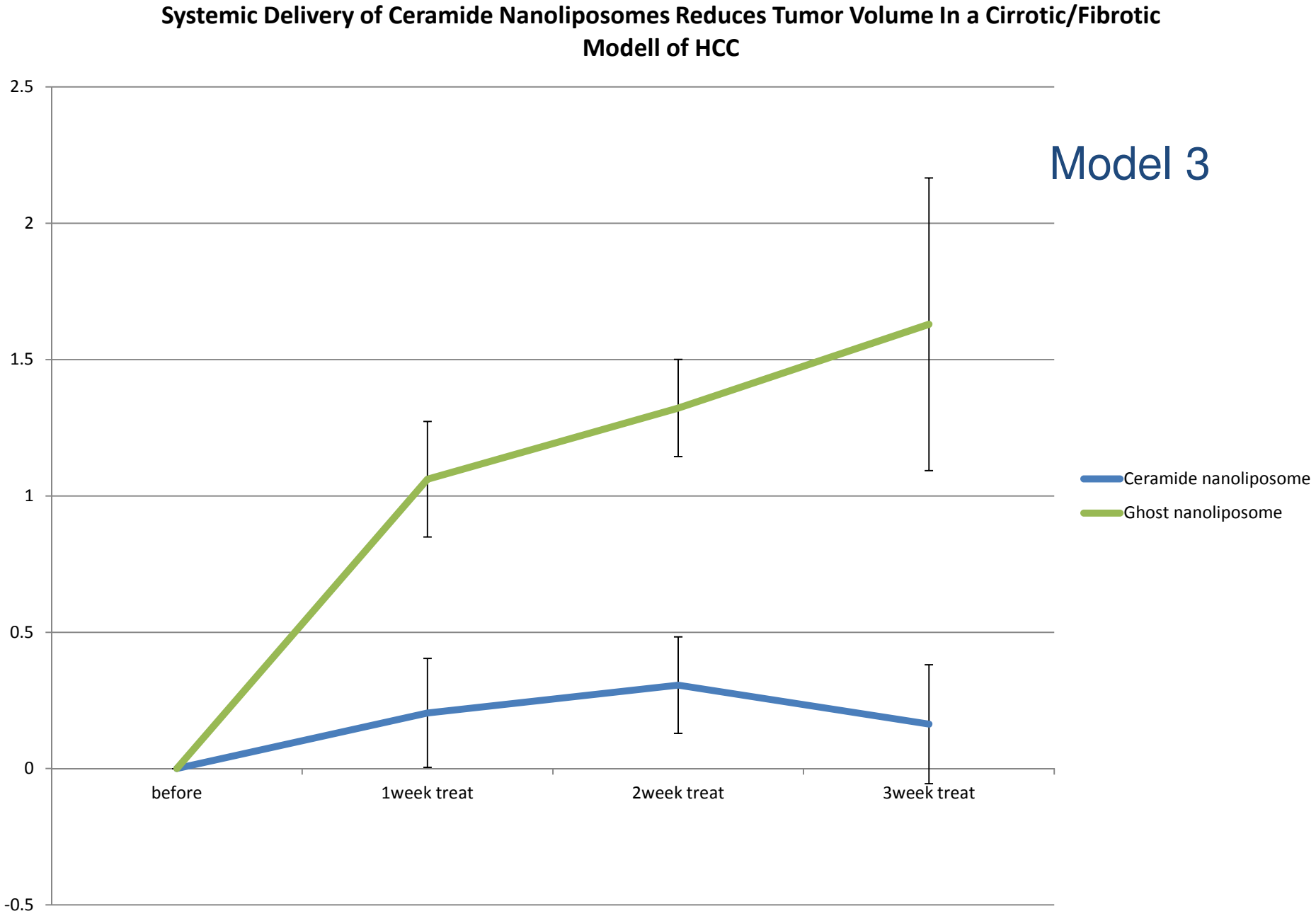


D



SK-HEP-1 cells treated with nanoliposomal C6-ceramide have diminished phosphorylation of AKT




Systemic Delivery of Ceramide Nanoliposomes Reduces Tumor Volume In a Cirrotic/Fibrotic Modell of HCC
Model 3
2.5
2
1.5
1
0.5
0
-0.5
before
1week treat
2week treat
3week treat
Ceramide nanoliposome
Ghost nanoliposome