UNIVERSITY OF MICHIGAN
COLLEGE OF PHARMACY

BIO INTERFACES INSTITUTE

Synthetic HDL –A Mimic of Nature's Nanomedicine

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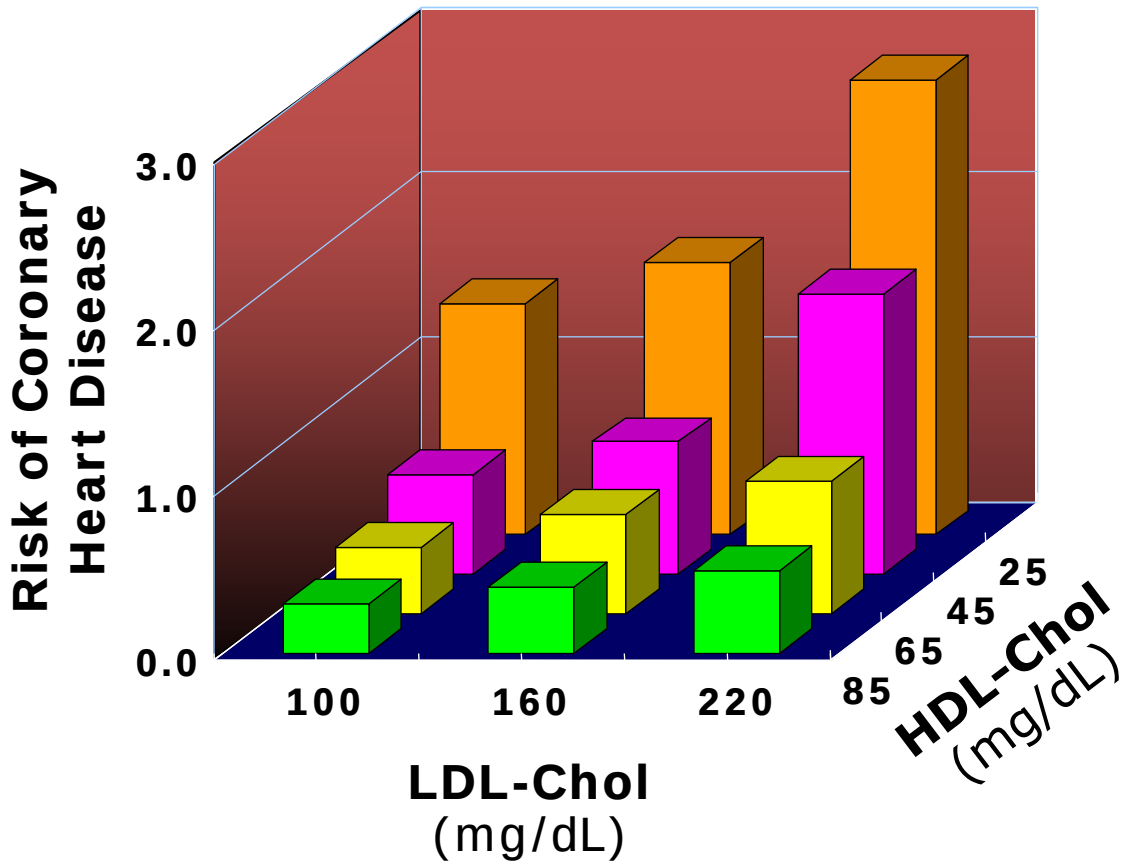
April 2017

Outline

- HDL and reverse cholesterol transport
- Optimizing sHDL for cholesterol mobilization
- LCAT-HDL molecular interaction and discovery of novel ApoA-I mimetic LCAT activators
- Application of sHDL for treatment of sepsis
- HDL nanodisc vaccine delivery platform for personalized treatment of cancer

Framingham Heart Study

Risk of Coronary Artery Disease in men 50-70 year old by HDL and LDL Cholesterol Levels



Source: AJC 2001; 88(suppl): 9N-13N

Lipoprotein Metabolism

www.colesterolbueno.com

Forward Cholesterol Transport

Apo B

LDL

"Bad Cholesterol"

Statins, Fibrates, Resins

Liver

Cholesterol Pool

SR-B1

Diet

Bile

Peripheral Cell

LDL-R

C

Cholesterol Pool

ABCA1

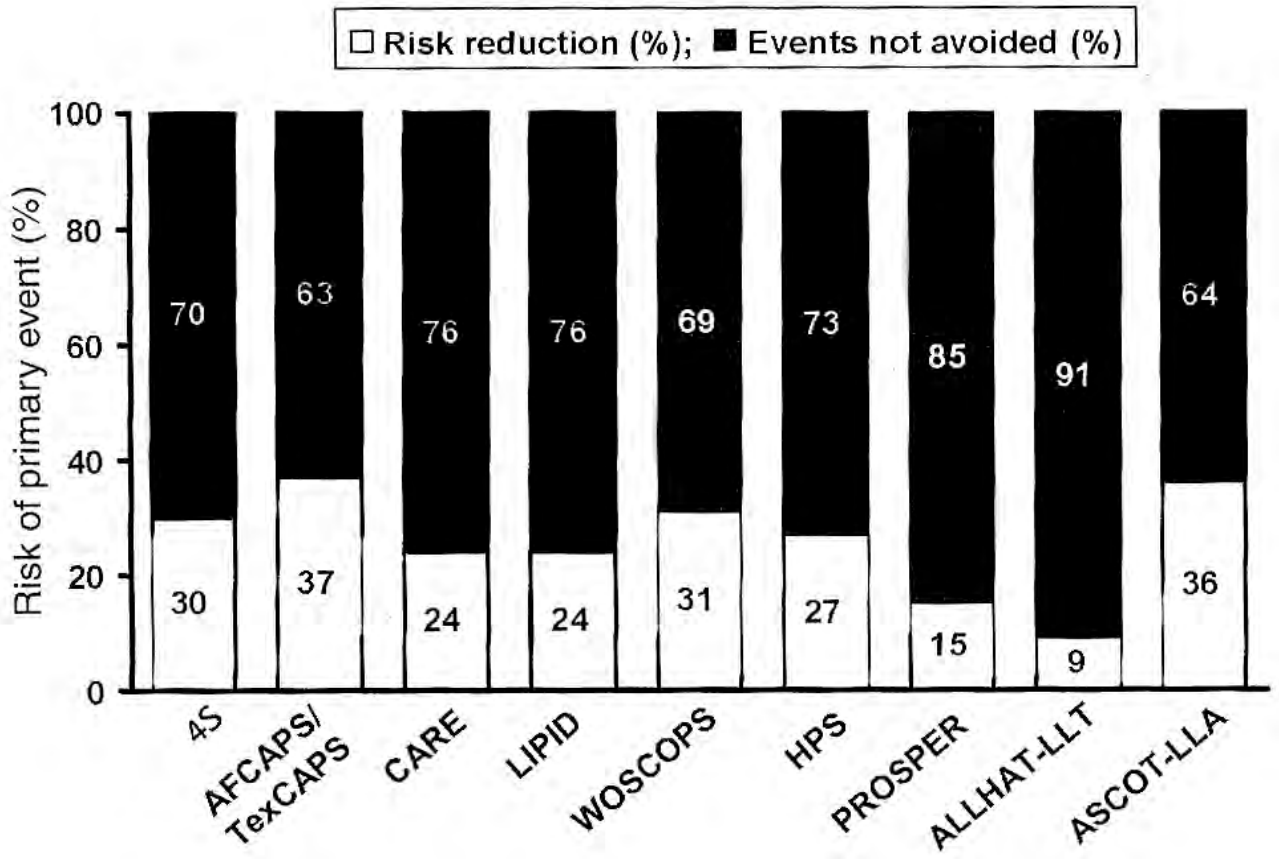
HDL

"Good Cholesterol"

Apo A-I

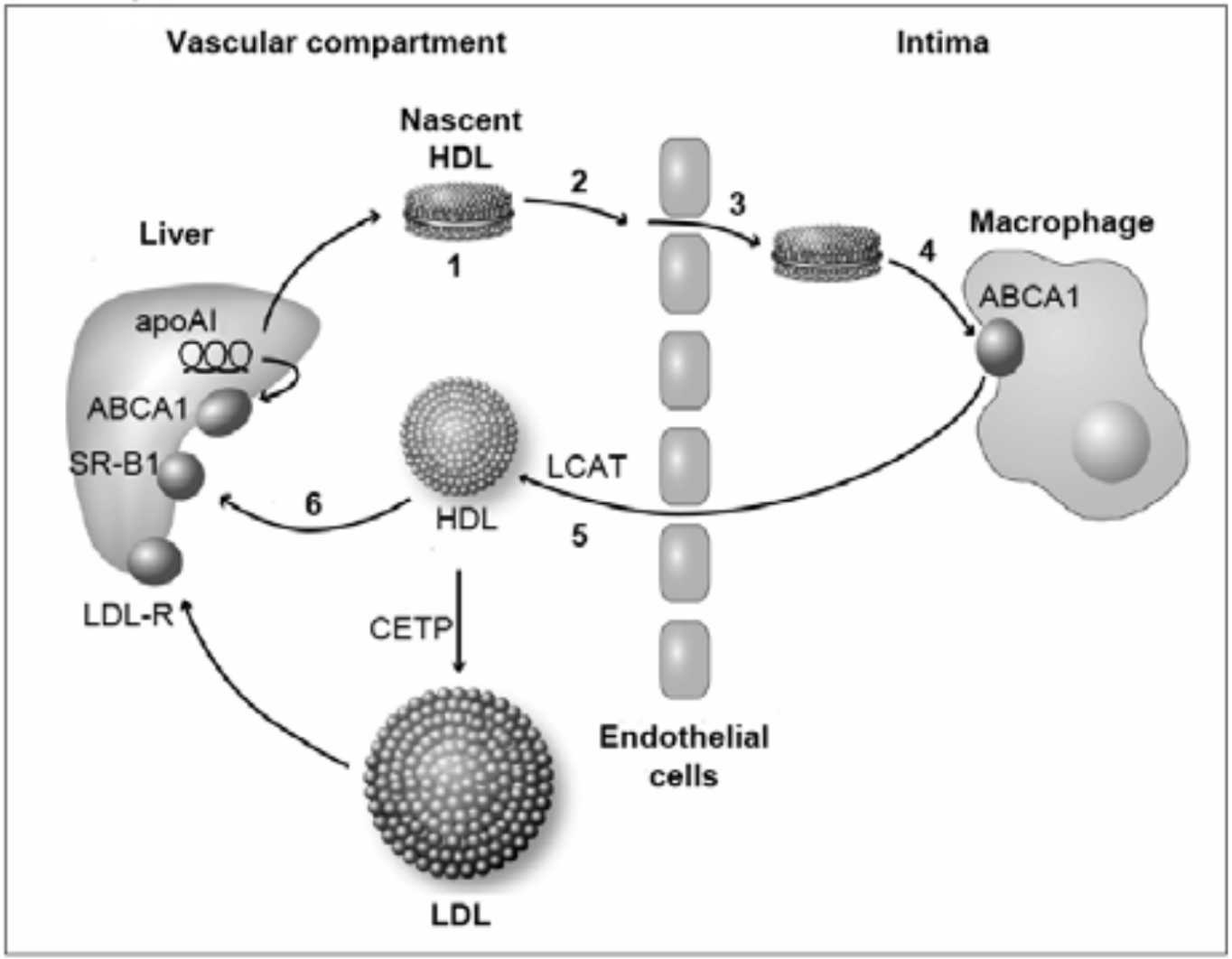
Reverse Cholesterol Transport

LDL Drugs – Modest Events Risk Reduction

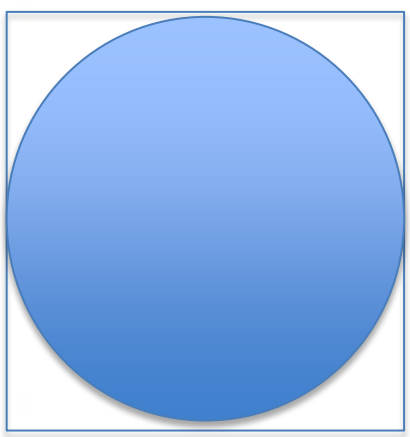


Kastelein. Eur Heart J 2005;7F:F27-F33.

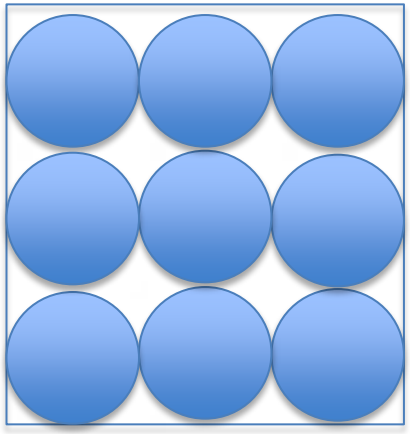
Reverse Cholesterol Transport



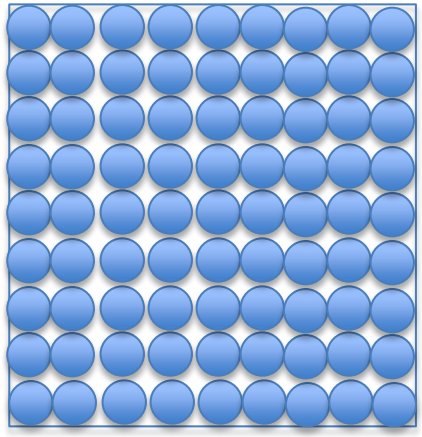
Why HDL is Important? – Lay Language



Exosome-VLDL
100-50 nm



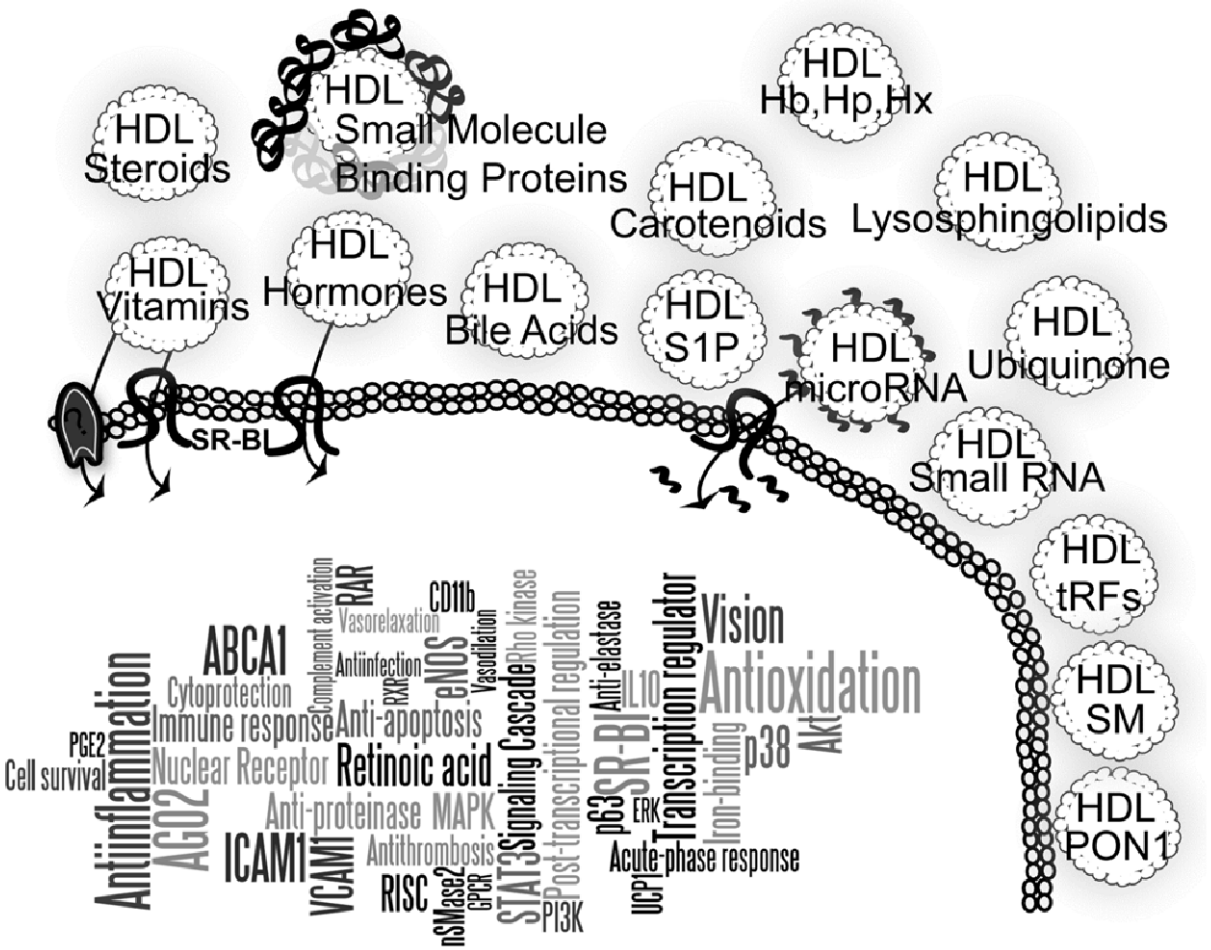
LDL
20 nm



HDL
8-10 nm

1. Oil and water don't mix
2. Small size – large number and surface area

HDL – scavenger and carrier of hydrophobic “stuff”

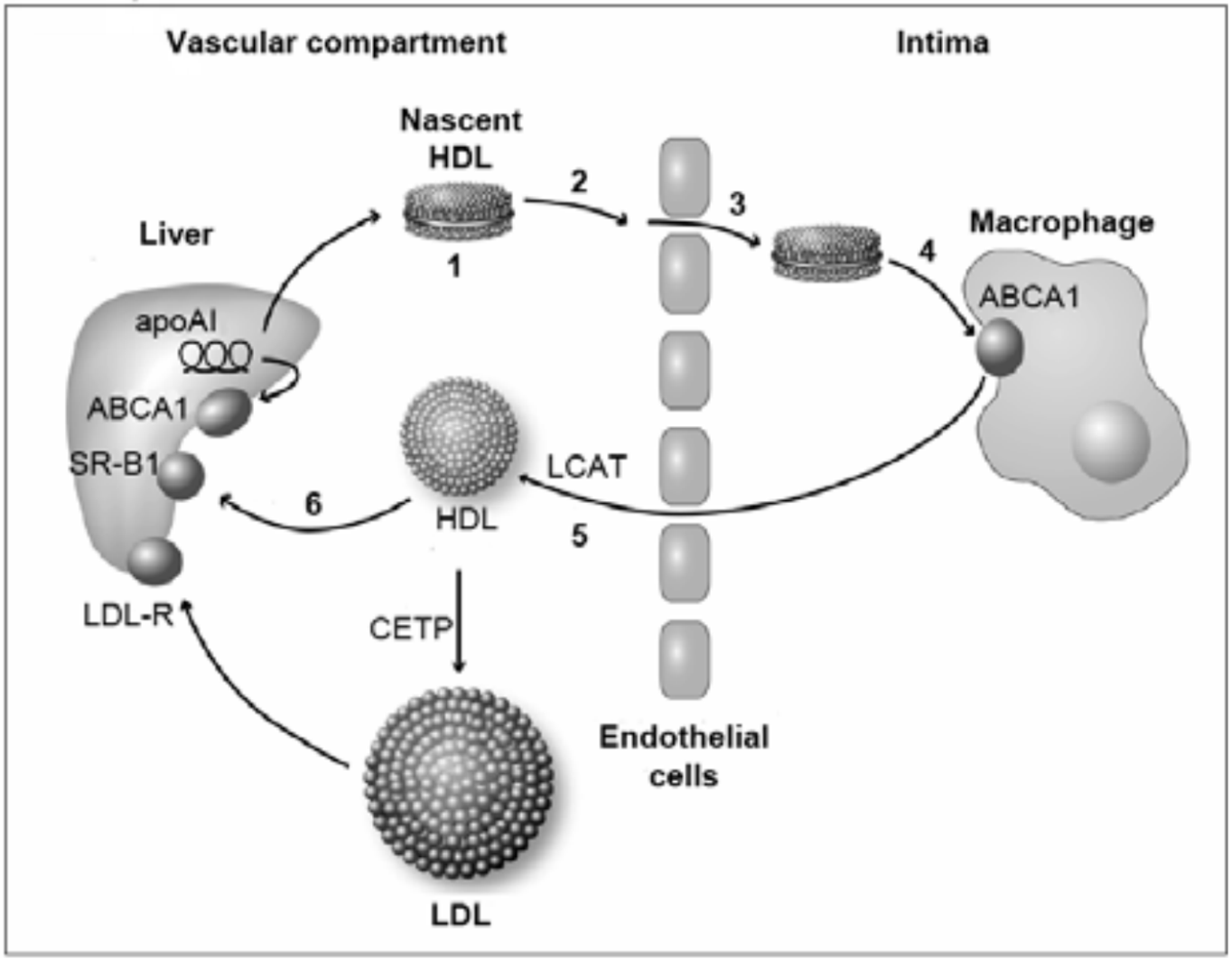


Remaley, Vickers, JLR, 2013

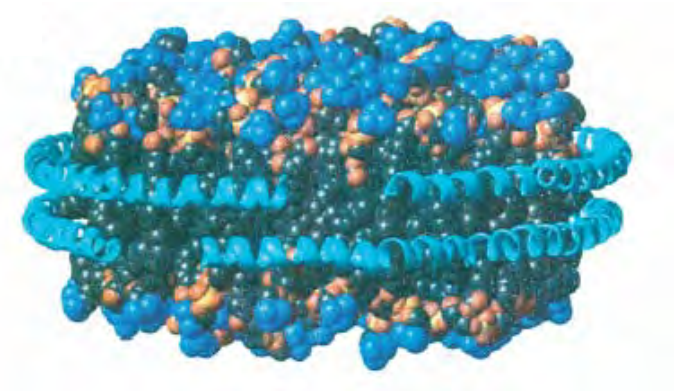
How HDL Really Looks Like…

Synthetic HDL – Arterial Drano®

Reverse Cholesterol Transport



HDL is a Nanoparticle

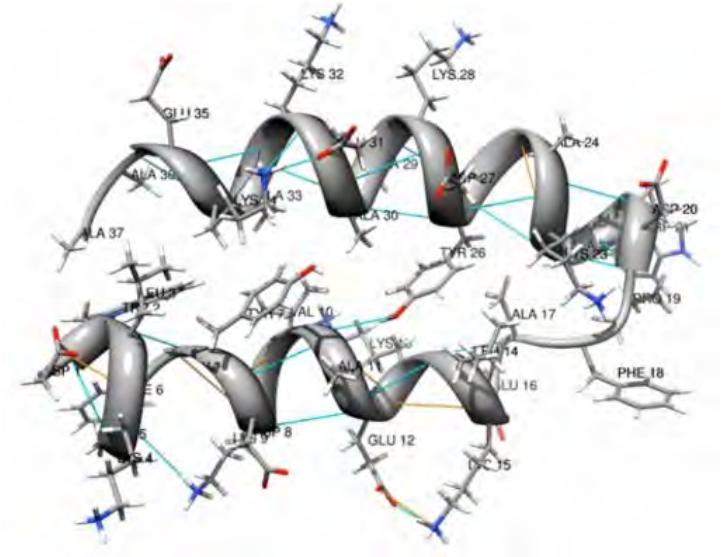


8-10 nm in diameter; 4 nm in thickness

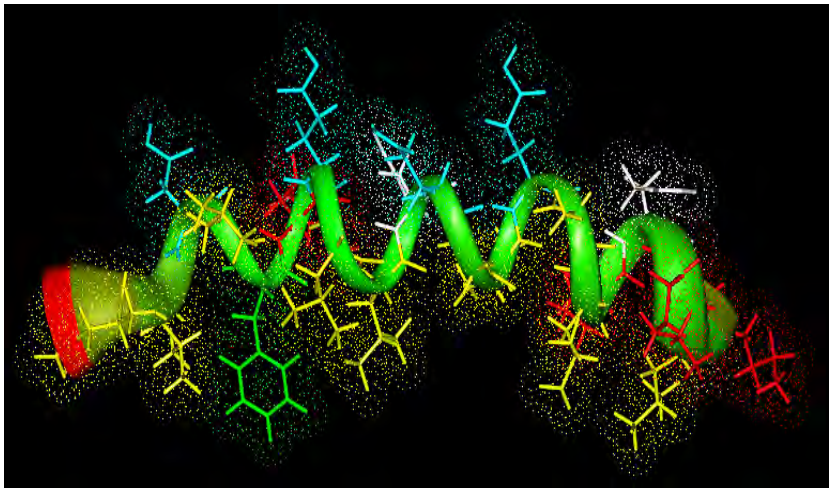
Lipid bilayer wrapped around by Apolipoprotein A-I

Protein Component of sHDL

Full length Apolipoprotein A-I



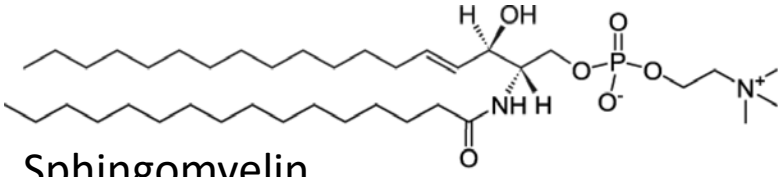
Bihelical 5A – ApoA-I Mimetic Peptide



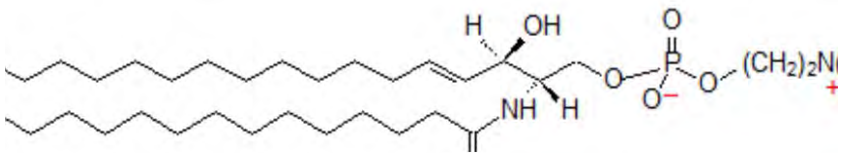
ETC-642 – Phase I Safety

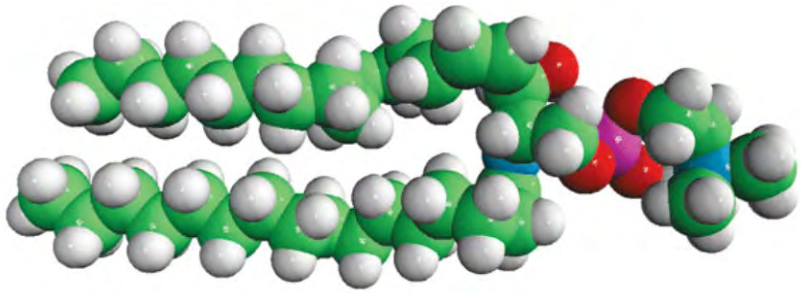
Synthetic HDL Lipid Components

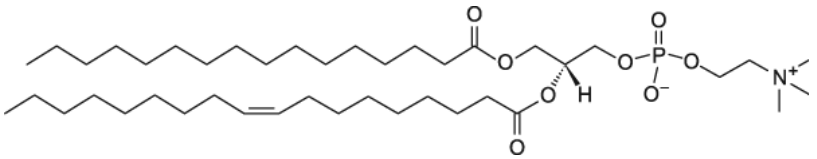
- Lipid type, fatty acid saturation and charge affects cholesterol efflux and plasma components binding
- Physical stability and plasma $t_{½}$ of HDL is affected



Sphingomyelin

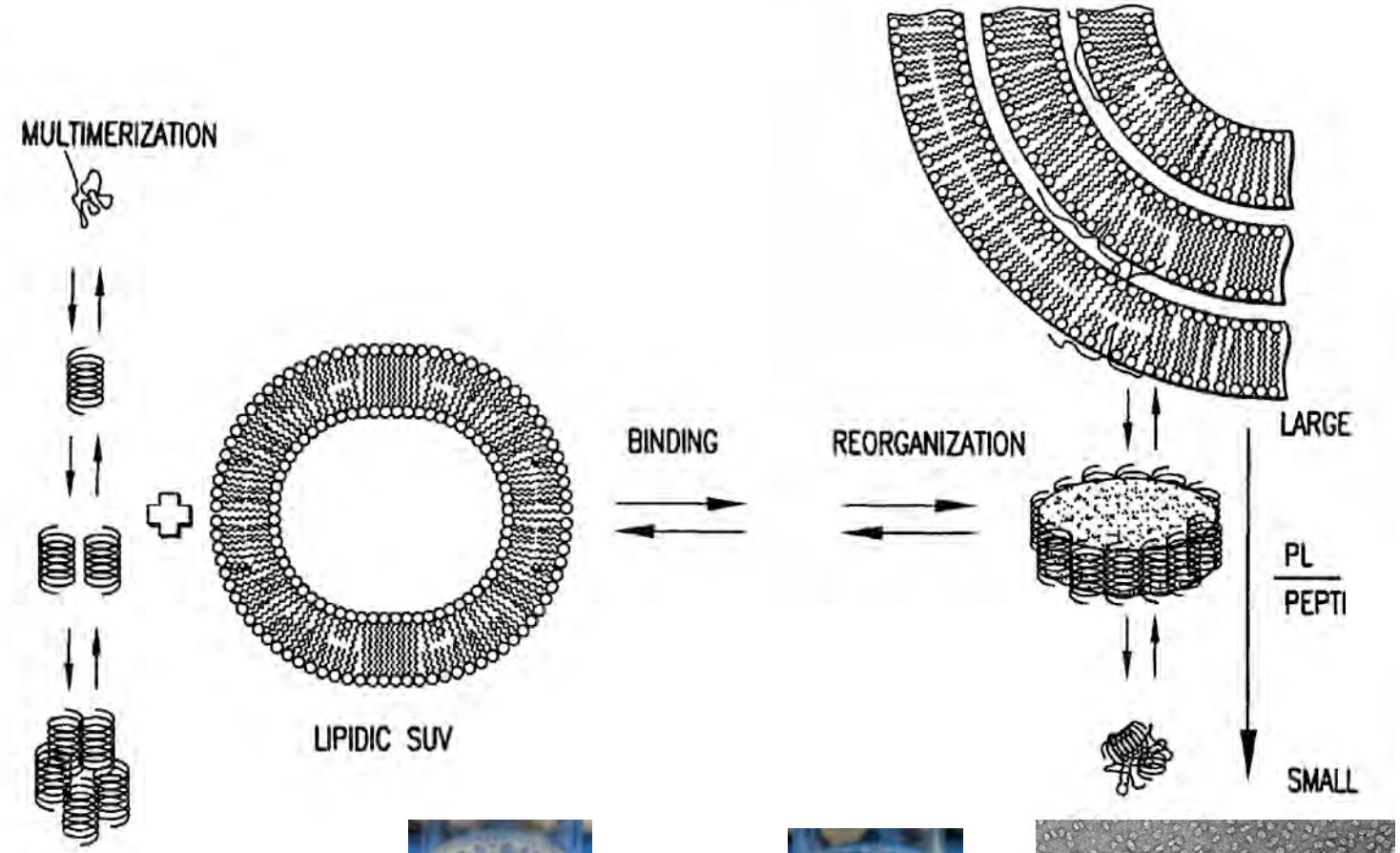


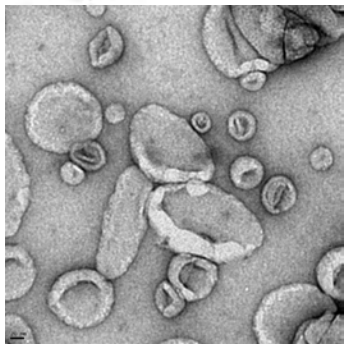


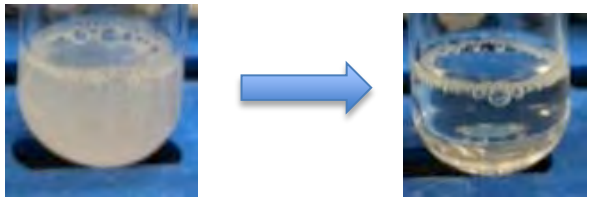


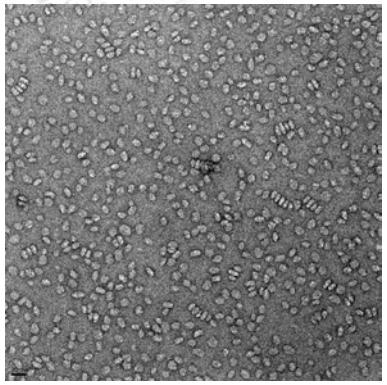
POPC

Dynamic of Synthetic HDL Formation

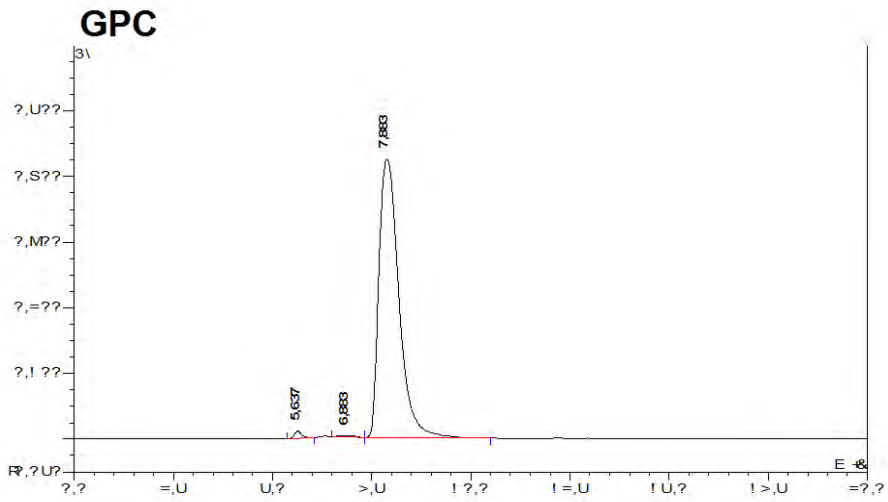


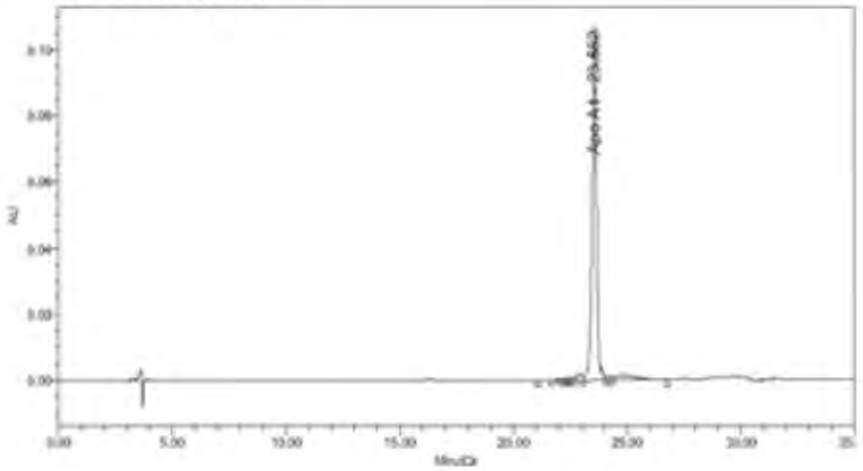


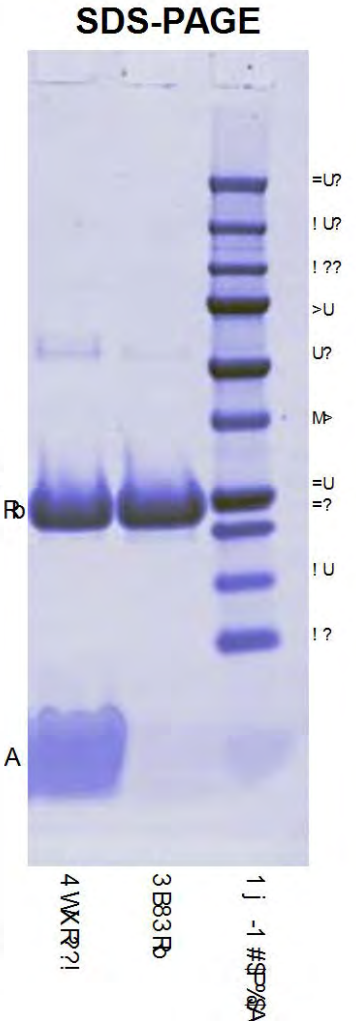




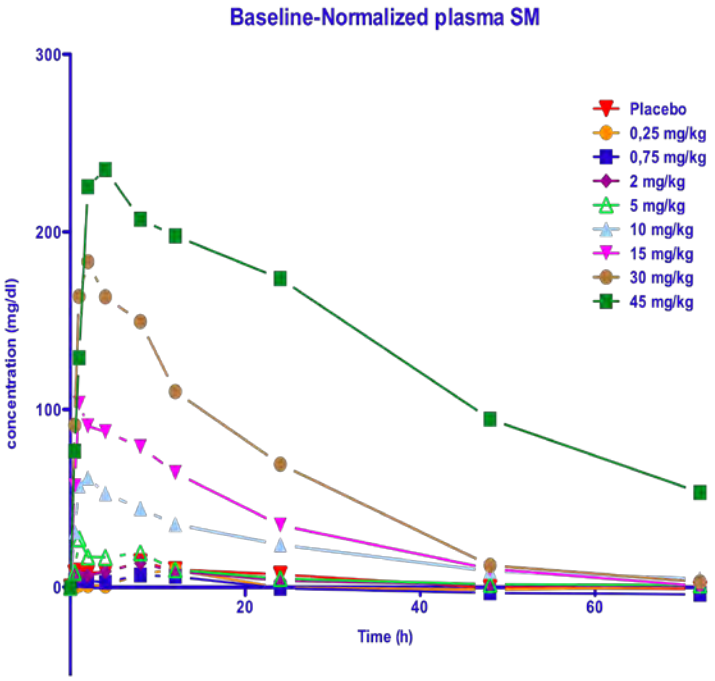
Making cGMP HDL

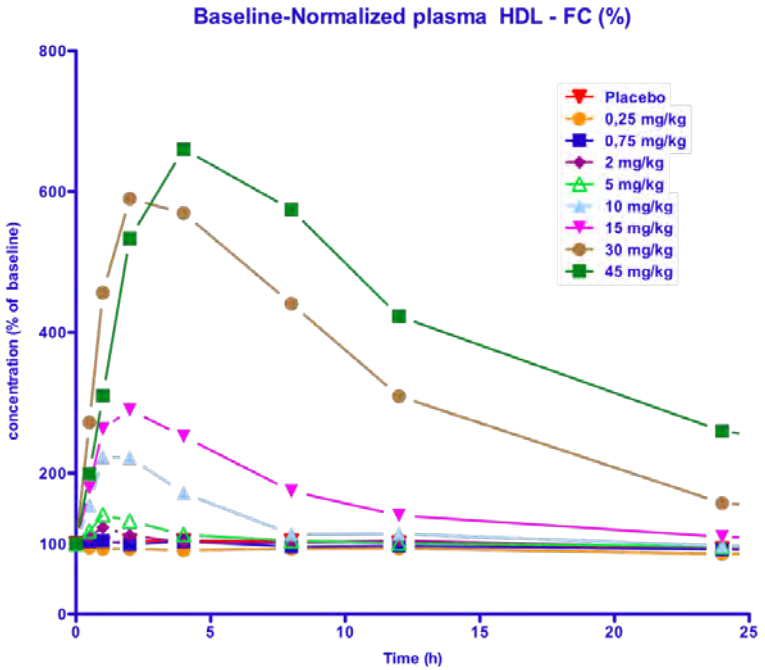






sHDL Safe and Mobilizes Cholesterol in People



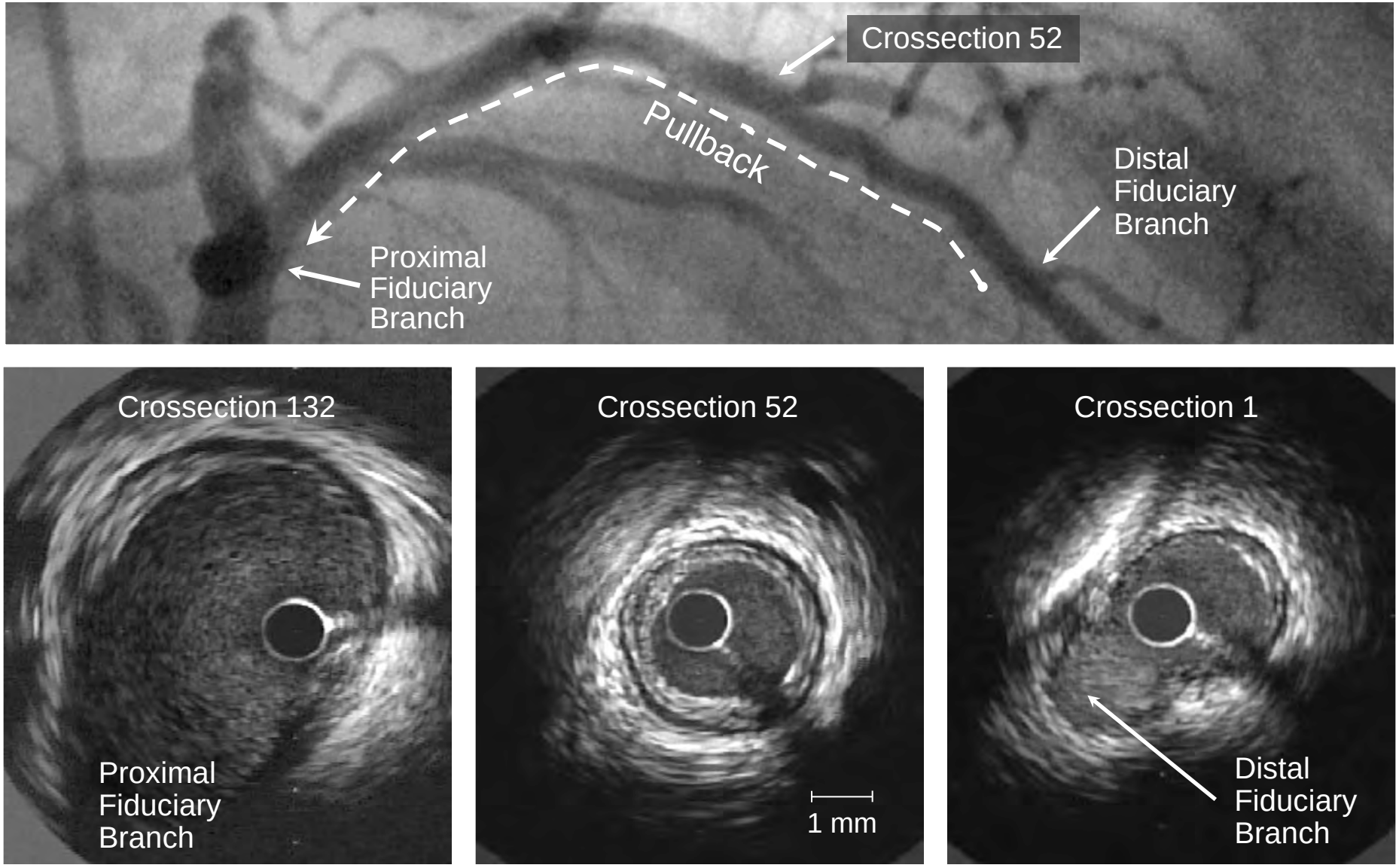


- **Safety at high doses (up to 45 mg/kg)**
- **Mobilization up to 7 times of baseline HDL cholesterol**
- **Circulates for 1-2 days**

Keyserling et al.
Circulation. 2011; 124: A15525

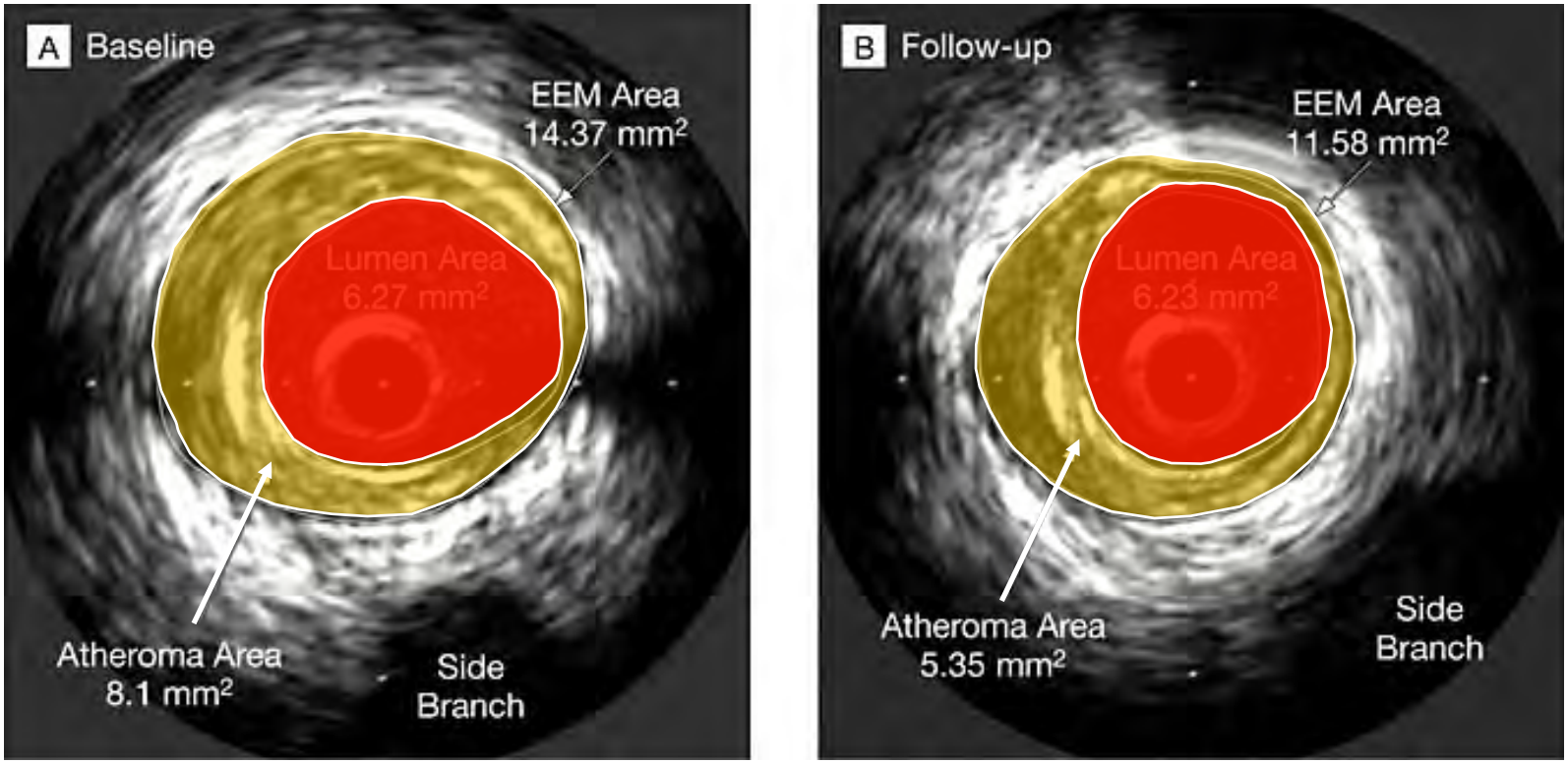
Intravascular Ultrasound Methodology

Pullback in target artery with cross-sectional slices each 0.5 mm



Nissen et al. JAMA 2003;290:2292-2300

sHDL Reduce Plaque Volume after 5 Infusions



Nissen et al. JAMA 2003;290:2292-2300

sHDL in Clinical Development

Name	Protein	Source	Studies/Development Stage
CSL-111	ApoA-I	Purified from plasma	Reduction or plaque by IVUS (Phase 2) Reduced inflammation/lipid content of plaque in PVD (Phase 2)
ETC-216	ApoA-I Milano	Recombinant	Reduction or plaque by IVUS (Phase 2)
ETC-642	ESP-24218 Peptide	Synthetic	Cholesterol mobilization and esterification in patients (Phase 1)
CER-001	ApoA-I	Recombinant	Mobilization cholesterol (Phase 1) Plaque reduction at low dose IVUS (Phase 2)
CSL-112	ApoA-I	Purified from plasma	Safety and cholesterol efflux (Phase 1) Large 1200 study (Phase 2)

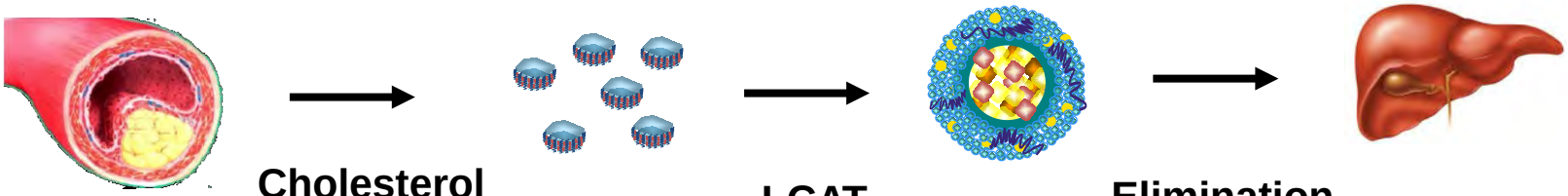
Main Issues with Protein-HDL Therapies

- Large doses are required
- Difficulties with production of homogeneous HDL nanoparticle (CSL-111 discontinuation)
- High manufacturing costs and toxicity associated with protein impurities (ETC-216 trial was halted)
- Poor clinical study designs, expansive IVUS imaging studies, high variability of data
- It not clear what lipid composition and dose is preferable (CER-001 IVUS failure for high/med doses)

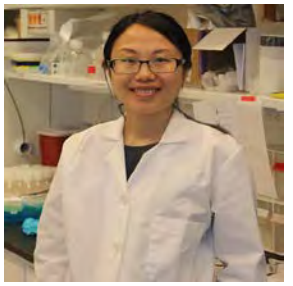
Alan Remaley

Eugene Chen

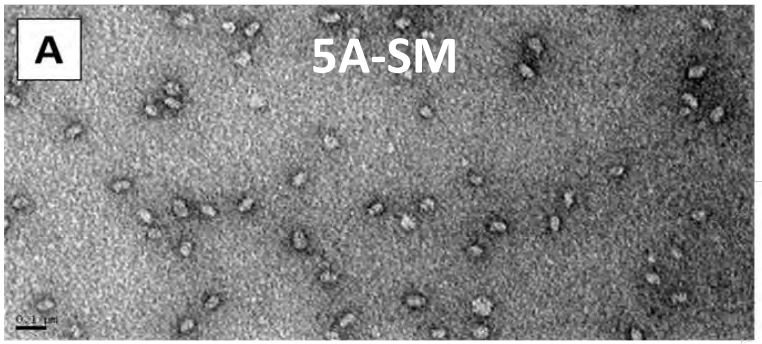
Optimization of sHDL Lipid Composition for Efflux and Elimination

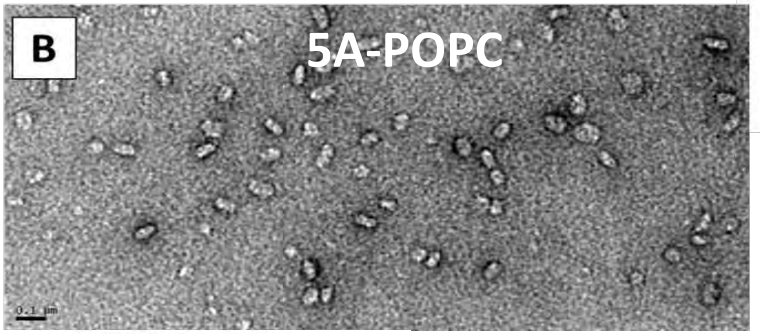


POPC and Sphingomyelin-Based SHDL

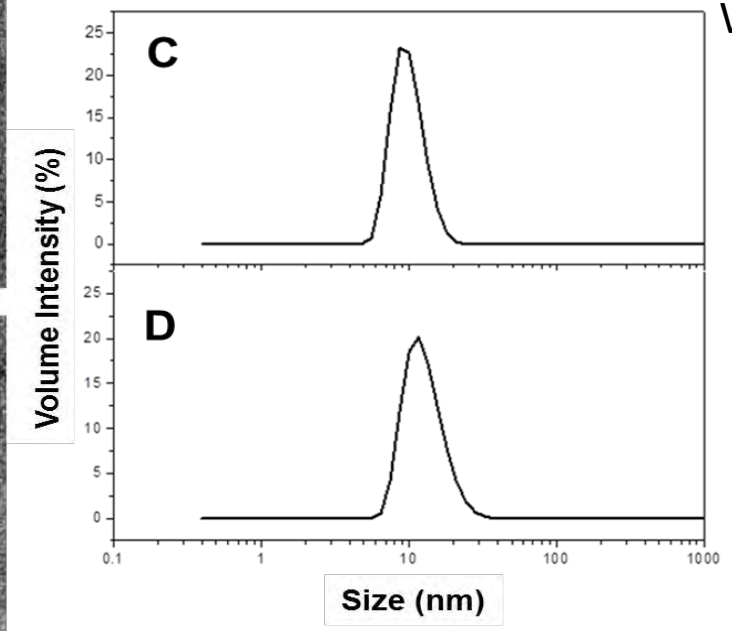


Wenmin Yuan

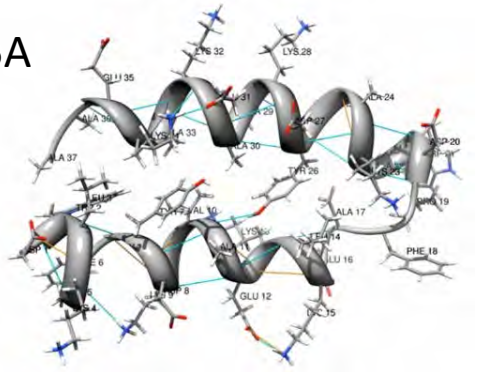


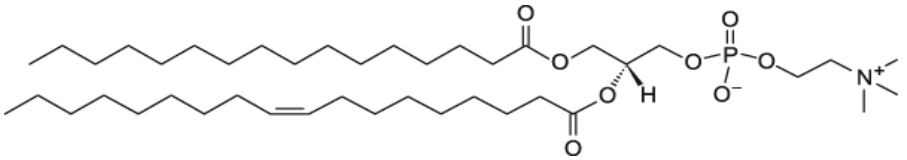


5A ApoA-I Mimetic Peptide

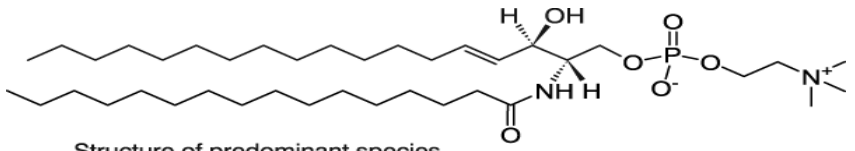


5A





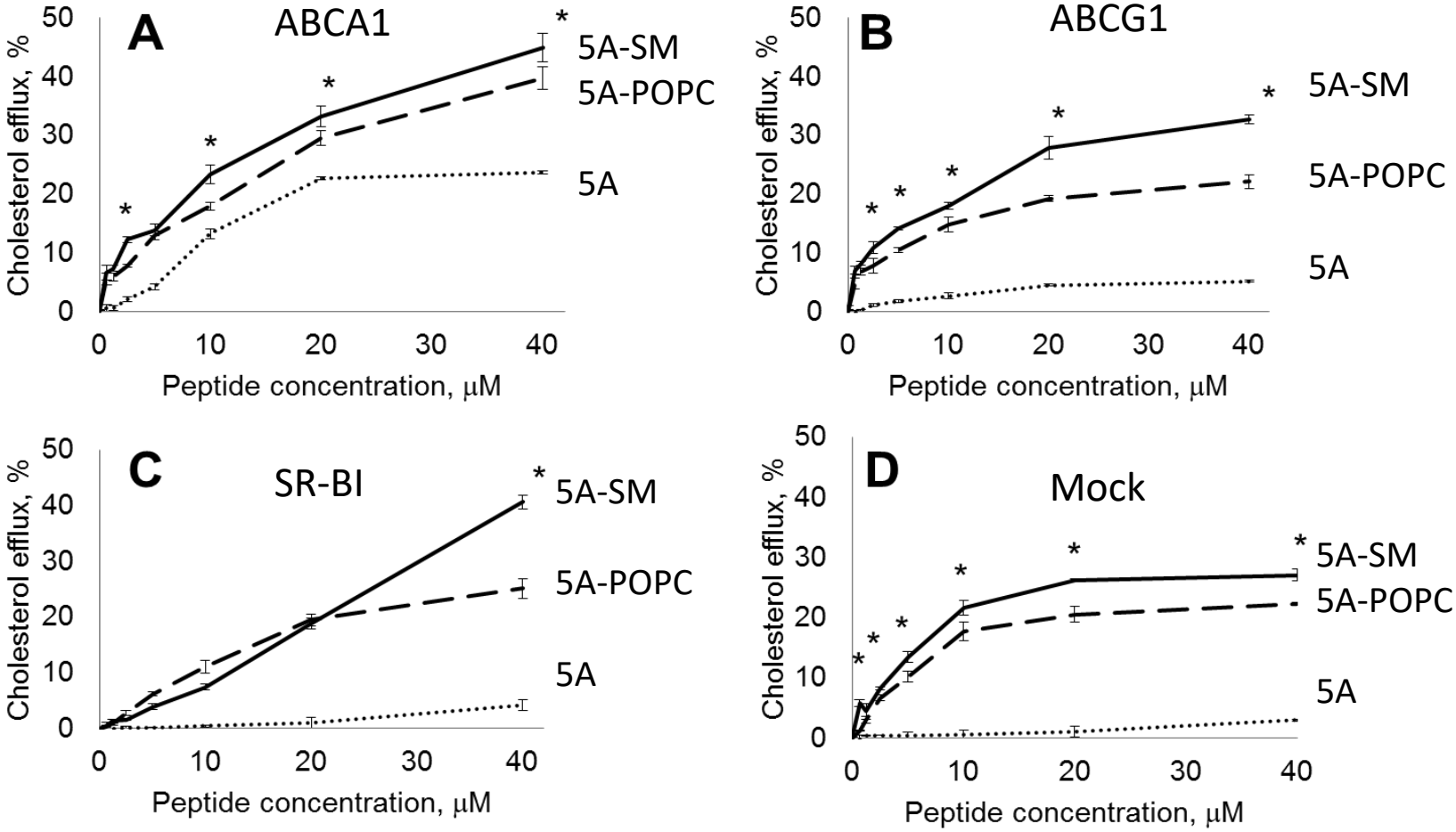
POPC



Structure of predominant species

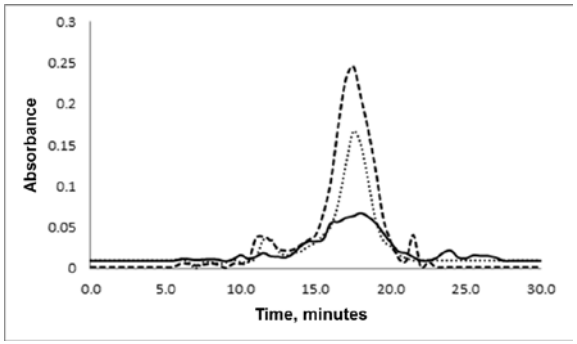
SM

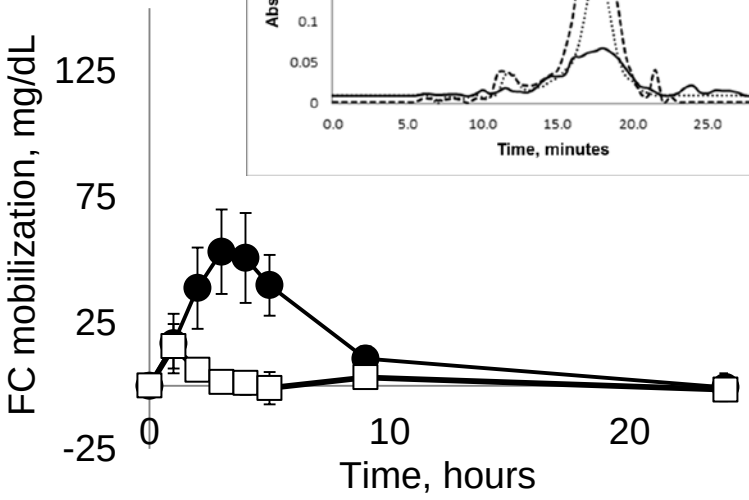
Cholesterol Efflux for POPC and SM-sHDL

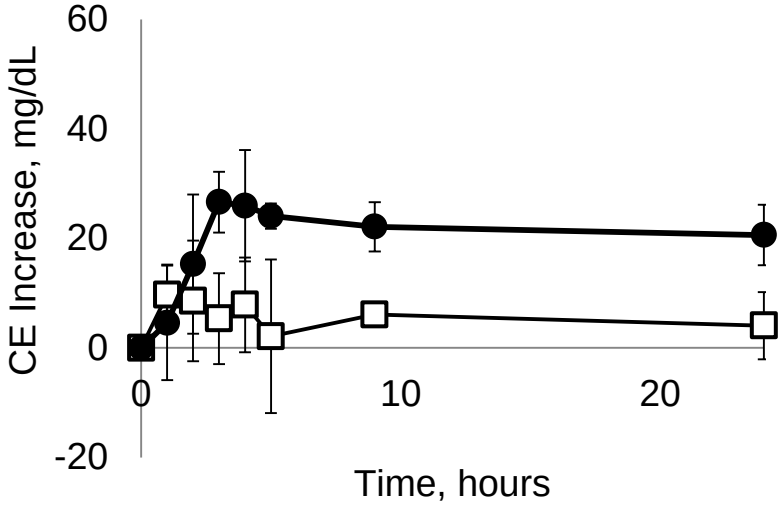


Higher Cholesterol Efflux for 5A-SM in vivo

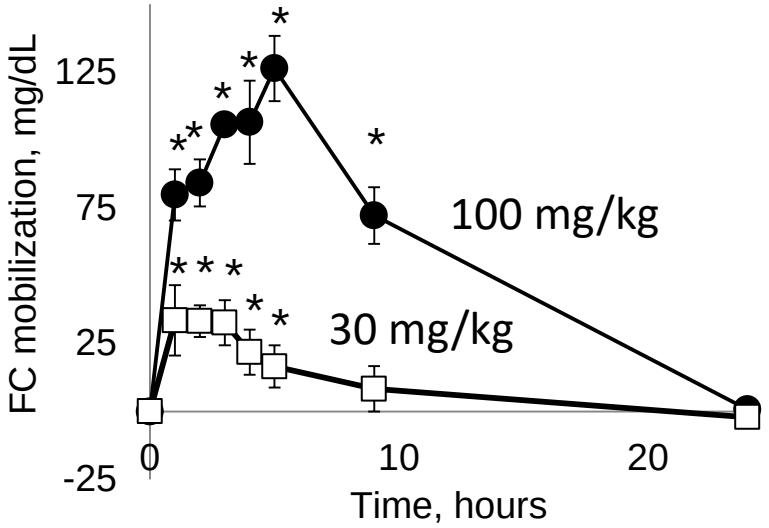
5A-POPC

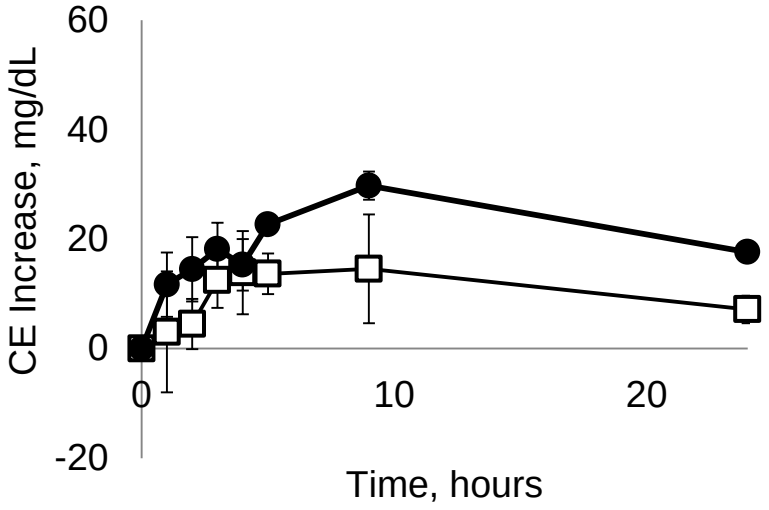




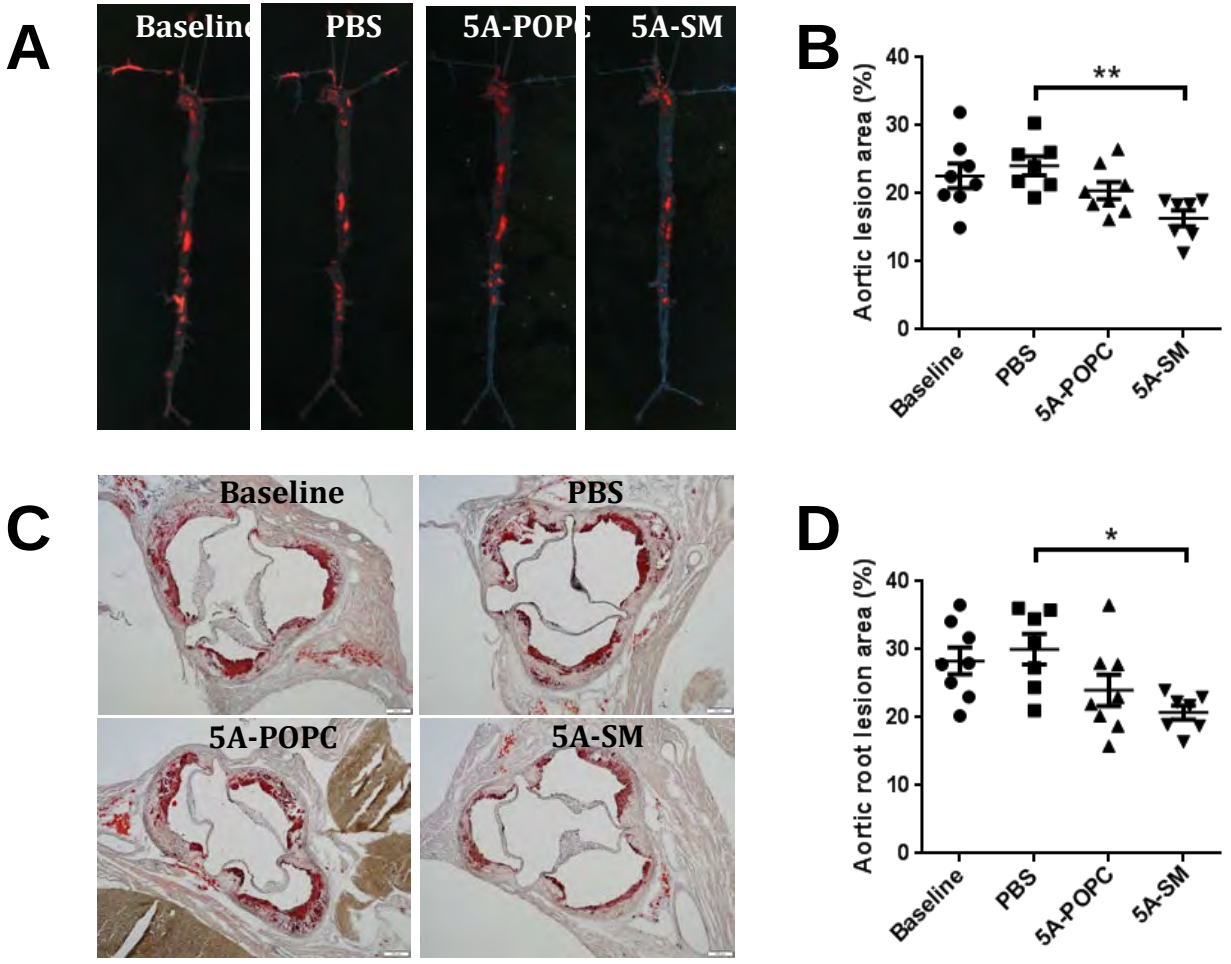


5A-SM





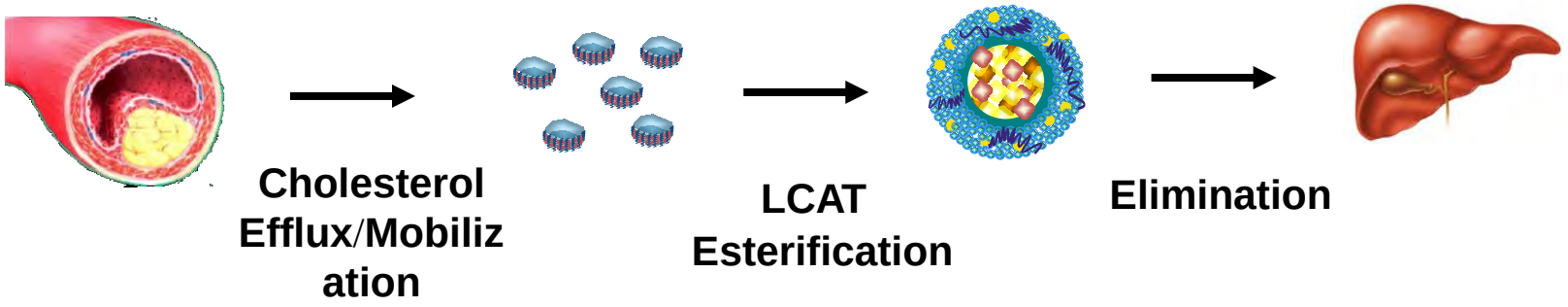
Stronger Anti-Atherosclerotic Effect in ApoE$^{-/-}$ for 5A-SM than 5A-POPC



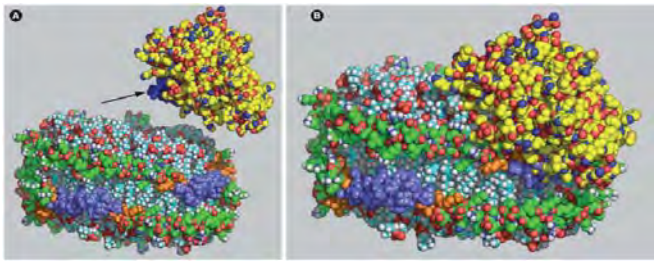
John Tesmer

Yiorgo Skiniotis,

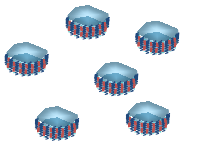
LCAT-HDL molecular interaction and discovery of novel ApoA-I mimetic LCAT activators



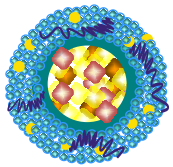
LCAT Molecular Interaction with HDL

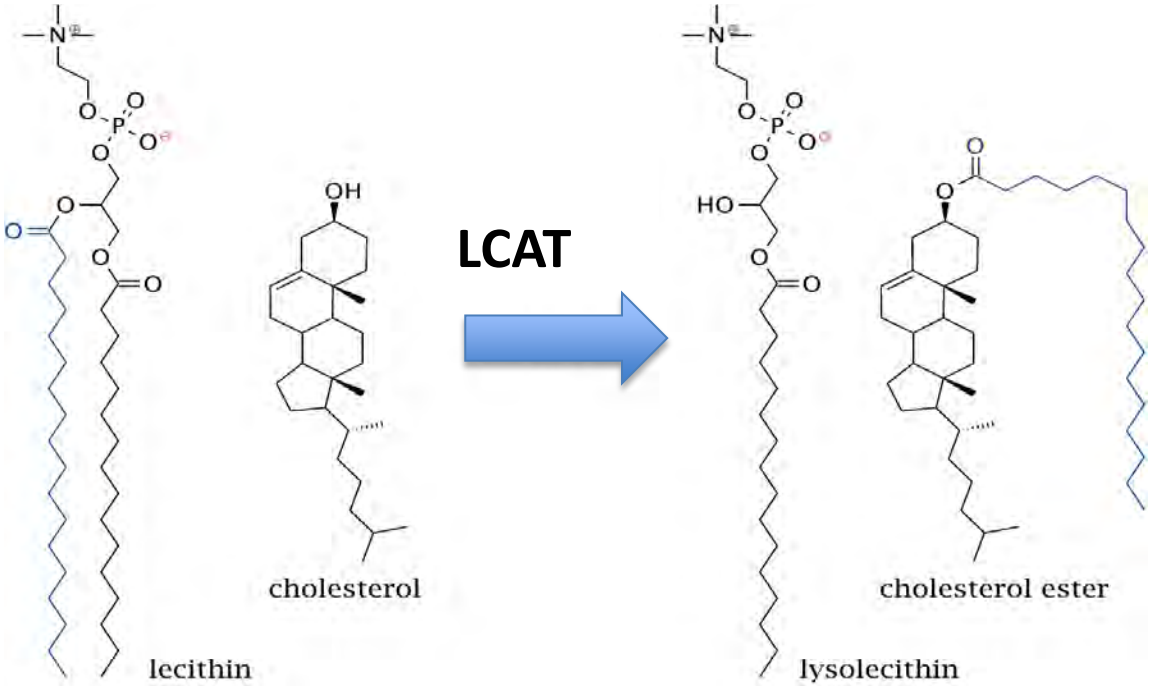


Sorci-Thomas et. al., Clin Lipidol, 2009

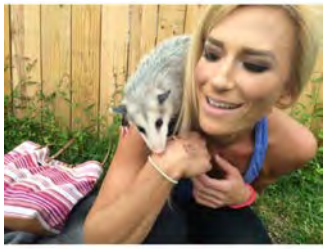


LCAT
Esterification



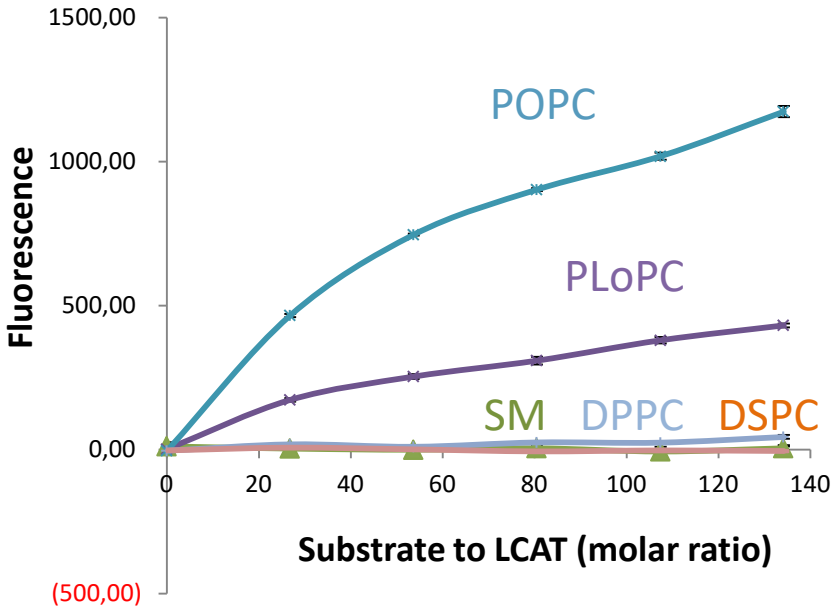


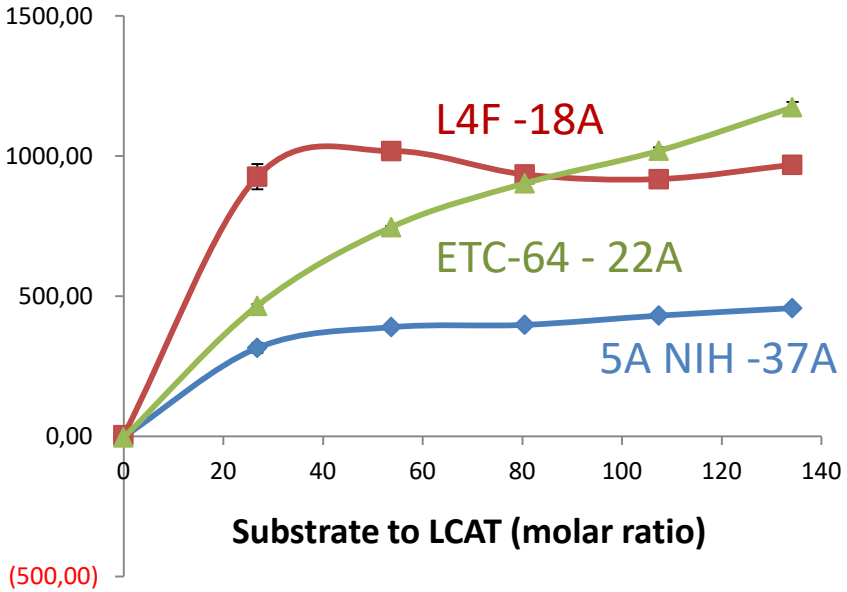
1. LCAT binds to HDL; **2.** Lipase activity; **3.** Transfer of acyl chain.



Jenny Shenkar

Optimizing HDL for LCAT activation

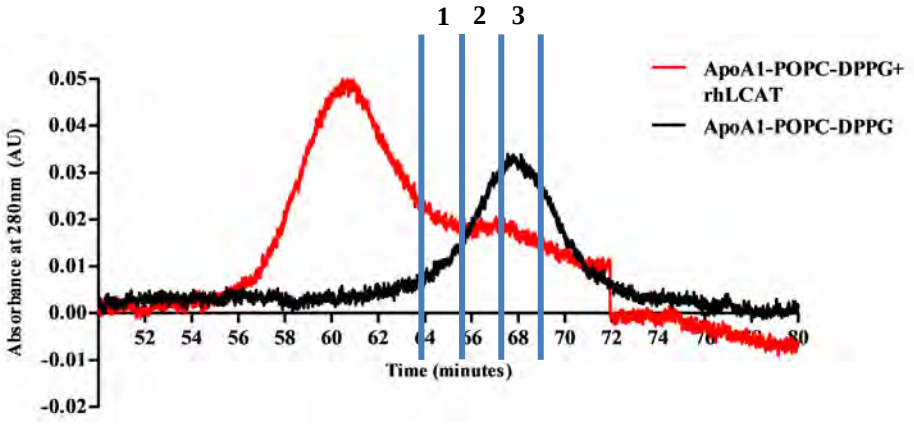


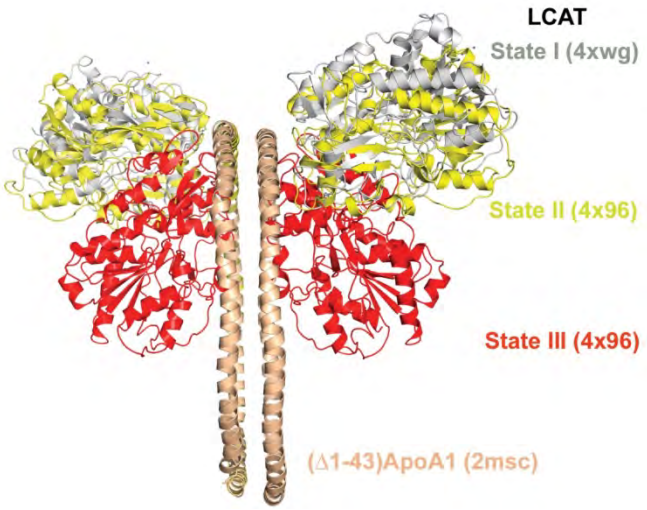


- Saturated lipids do not appear to be substrates for LCAT
- LCAT and ApoA-I peptide molecular interaction

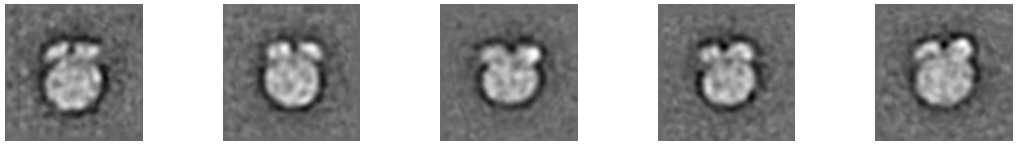
AstraZeneca

Isolation and Imaging of LCAT-HDL Fraction

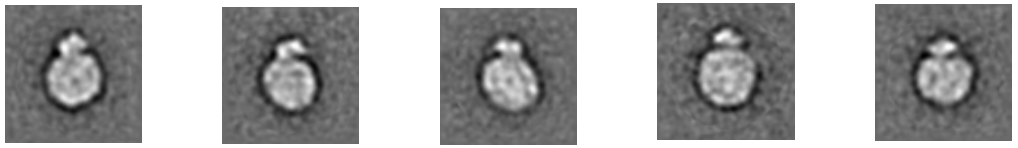




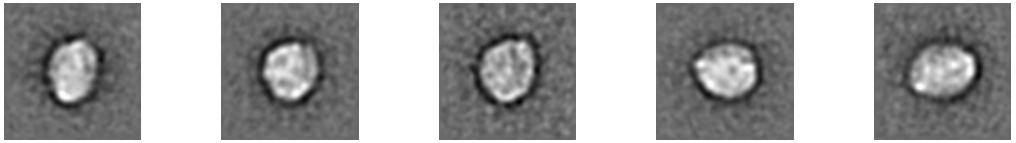
2 LCAT-HDL (16%)



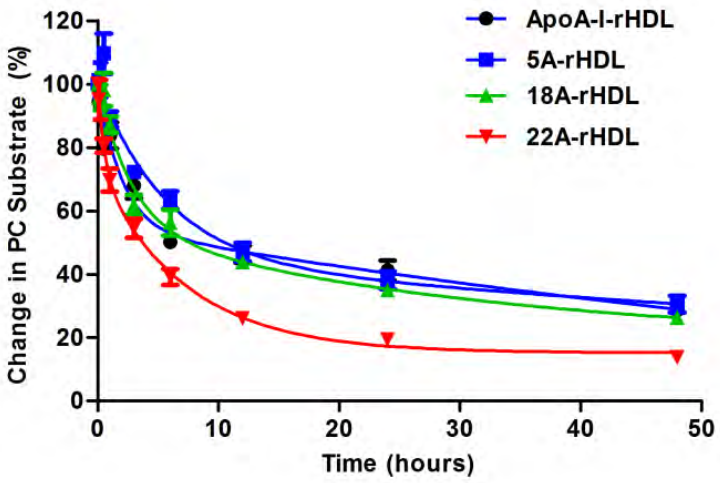
1 LCAT-HDL (58%)

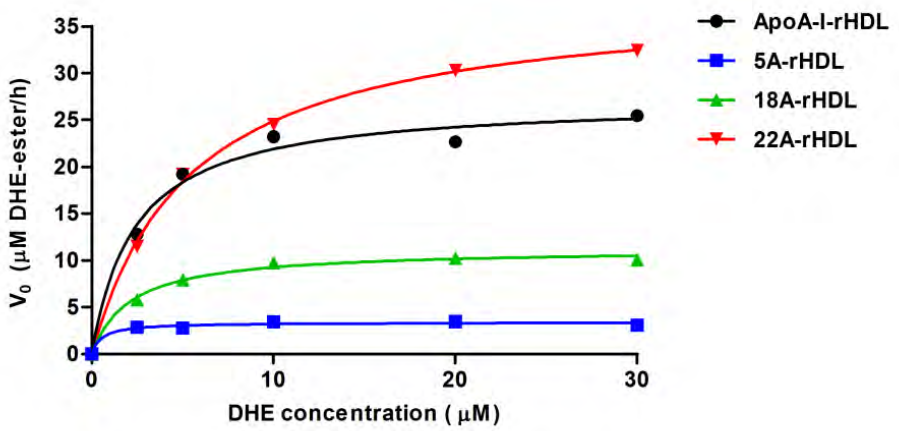


HDL 641 (26%)



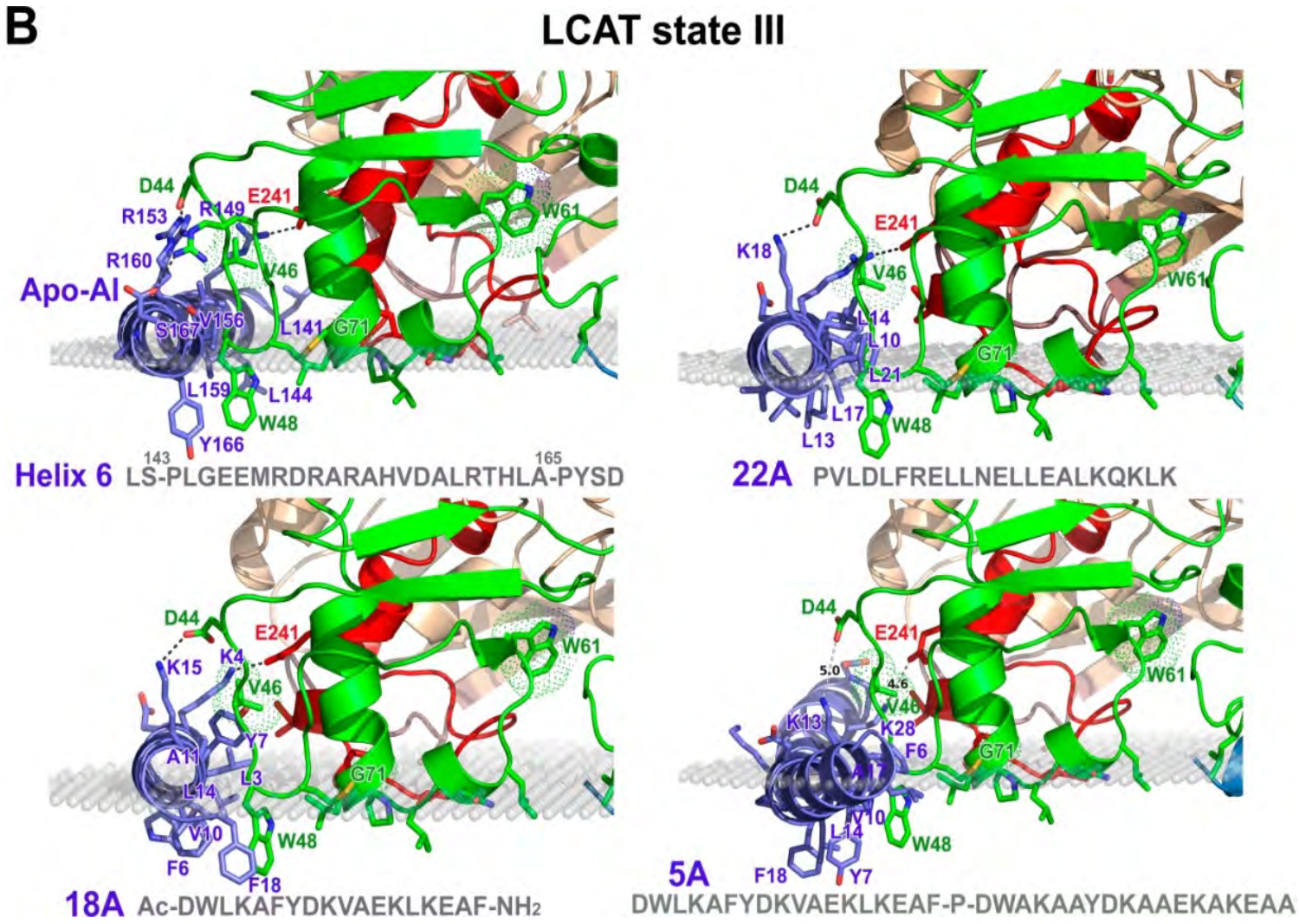
Molecular Interaction of LCAT and ApoA-I Peptides





Peptide	ΔG_{helix}, kcal/mol	$\Delta G_{transfer}$, kcal/mol	D, Å	Vmax (uM DHE ester/h)	Kcat, min^{-1}	Lipolysis rate ($t_{1/2}$)
22A	-24.3	-16.9	7.8	38.34	15.8	0.94
18A	-20.1	-12.7	8.2	11.24	4.89	0.93
5A	-13.3	-9.3	7.6	3.37	1.86	2.71

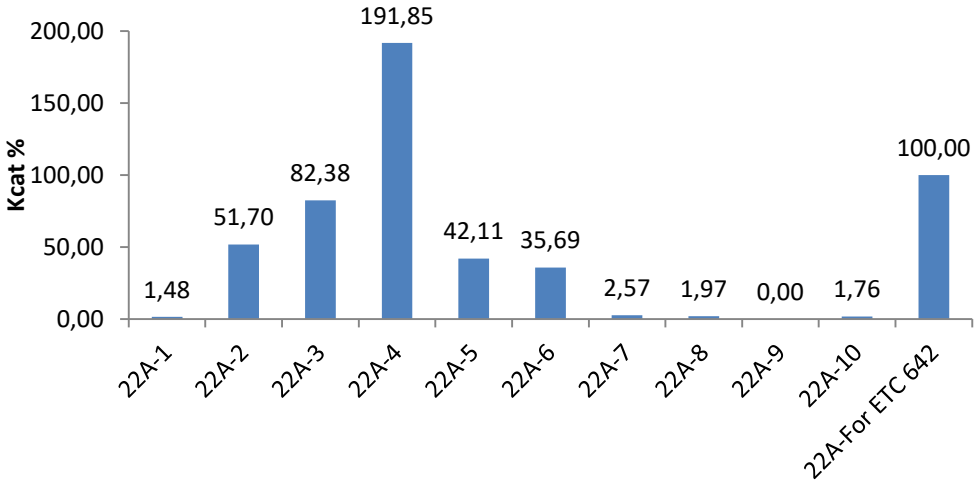
ApoA-I Peptides and Activated LCAT Molecular Interactions



HDL Formation and LCAT Activity of ApoA-I Mimetics

Freshly made

After Stored at 4 degree for 1 week



	22A-1	22A-2	22A-3	22A-4	22A-5	22A-6	22A-7	22A-8	22A-9	22A-10	22A-For ETC 642
Vmax (µM DHE ester/h)	0.52	18.28	29.13	67.84	14.89	12.62	0.91	0.70	NA	0.62	35.37
Km (µM DHE)	NA	2.00	6.10	5.70	9.02	4.20	0.73	0.29	NA	3.62	4.42
kcat (/min)	0.23	8.17	13.02	30.31	6.65	5.64	0.41	0.31	NA	0.28	15.80

HDL in Sepsis – Risk Factor and Potential Therapy

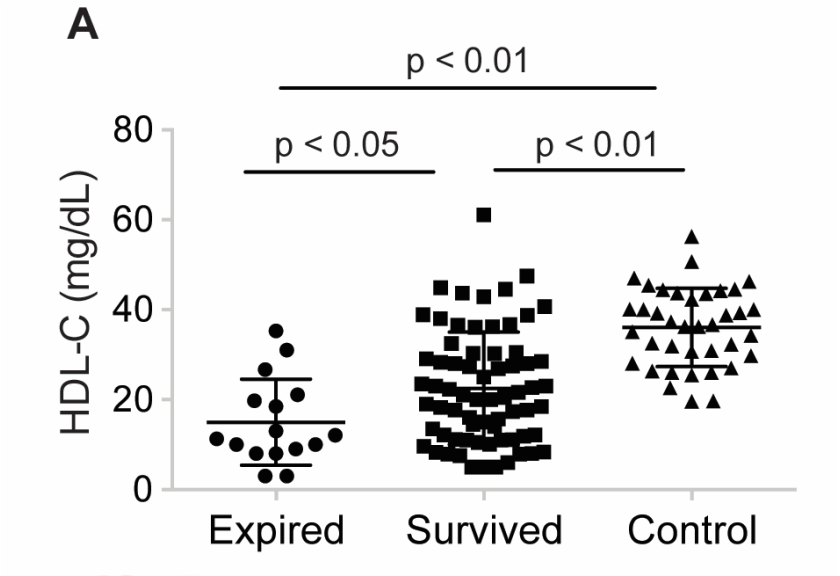
Xiang-An Li, PhD

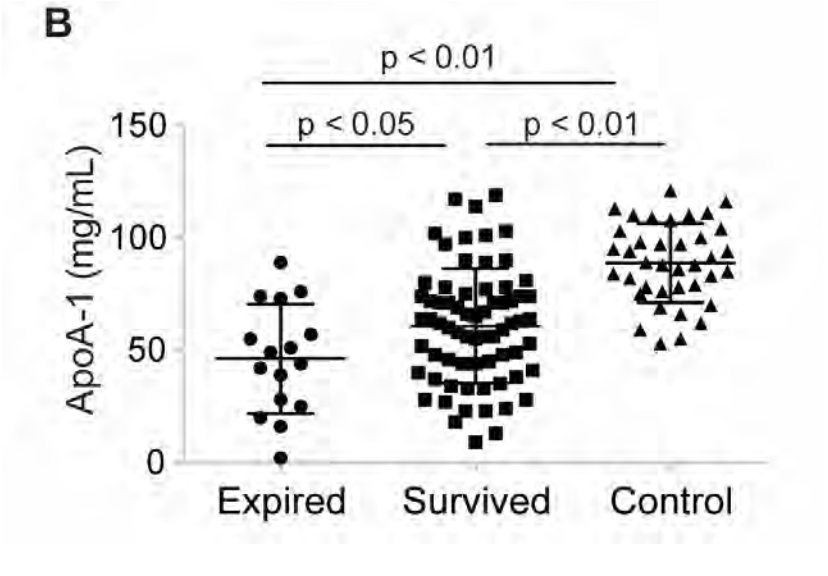
Ted Standiford, MD

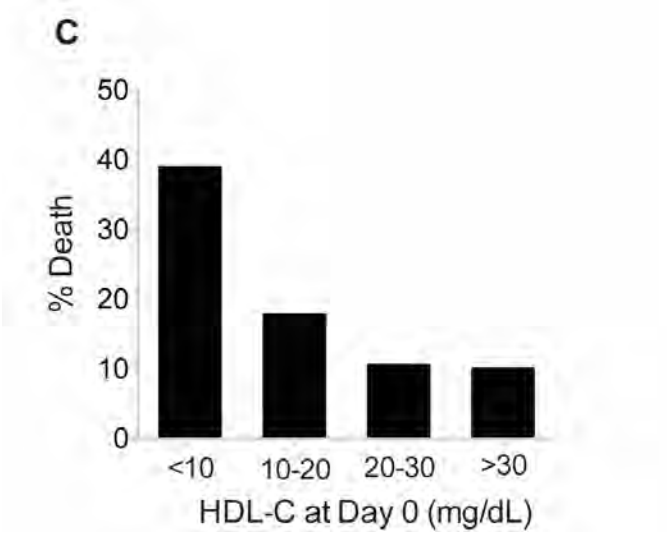
Reasons for Using sHDL to Treat Sepsis

- Endotoxin physically binds to ApoA-I and HDL
- Evidence of endothelial function improvement in patients
- There are no approved drugs for sepsis
- Total hospitalizations 1.4M and 250,000 death/year in US
- $14-17B spent on sepsis care
- Billion dollar “bench to bedside” commercial opportunity

Rapid 50-70% HDL-C and ApoA-I Drop in Sepsis

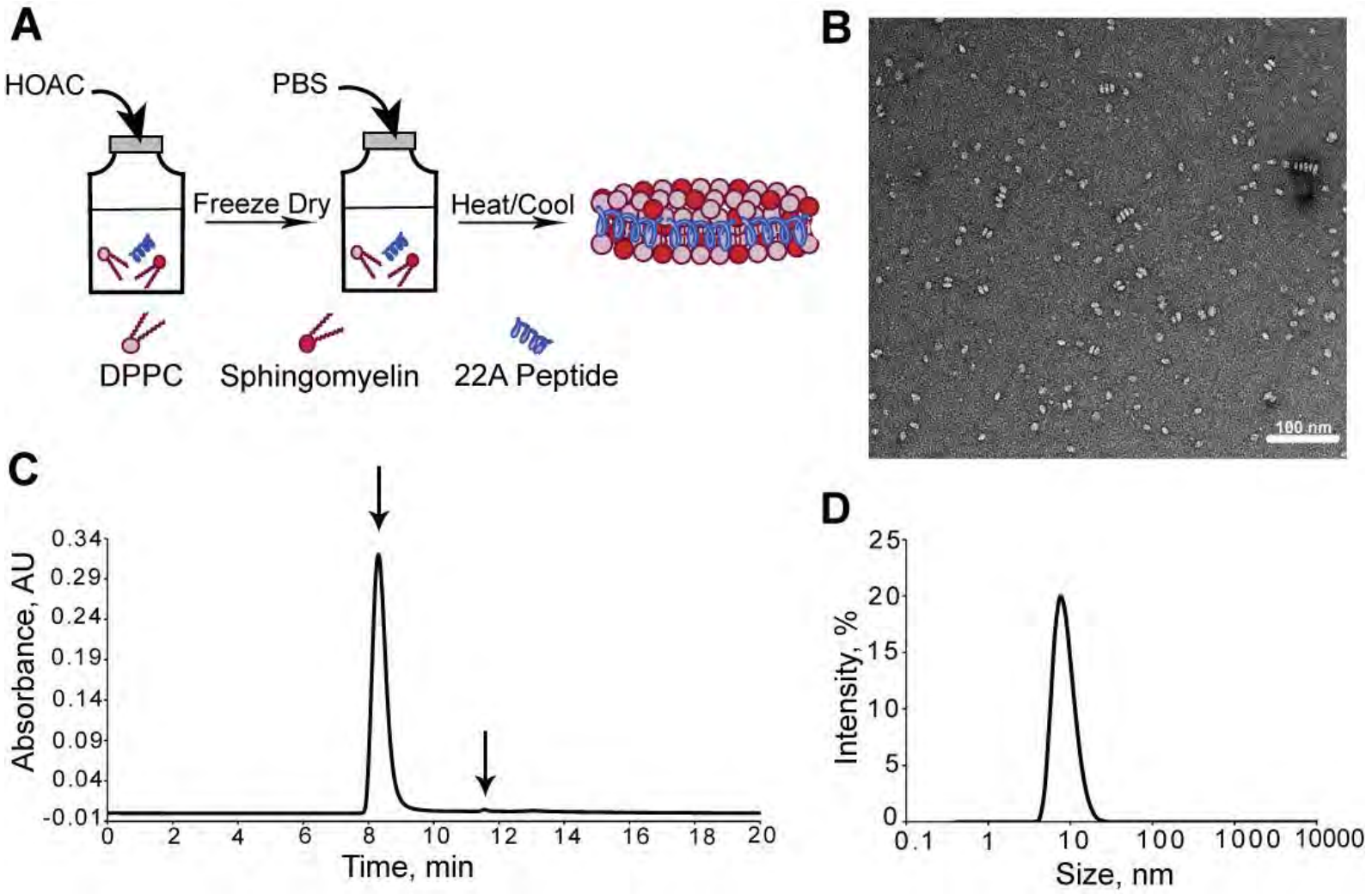




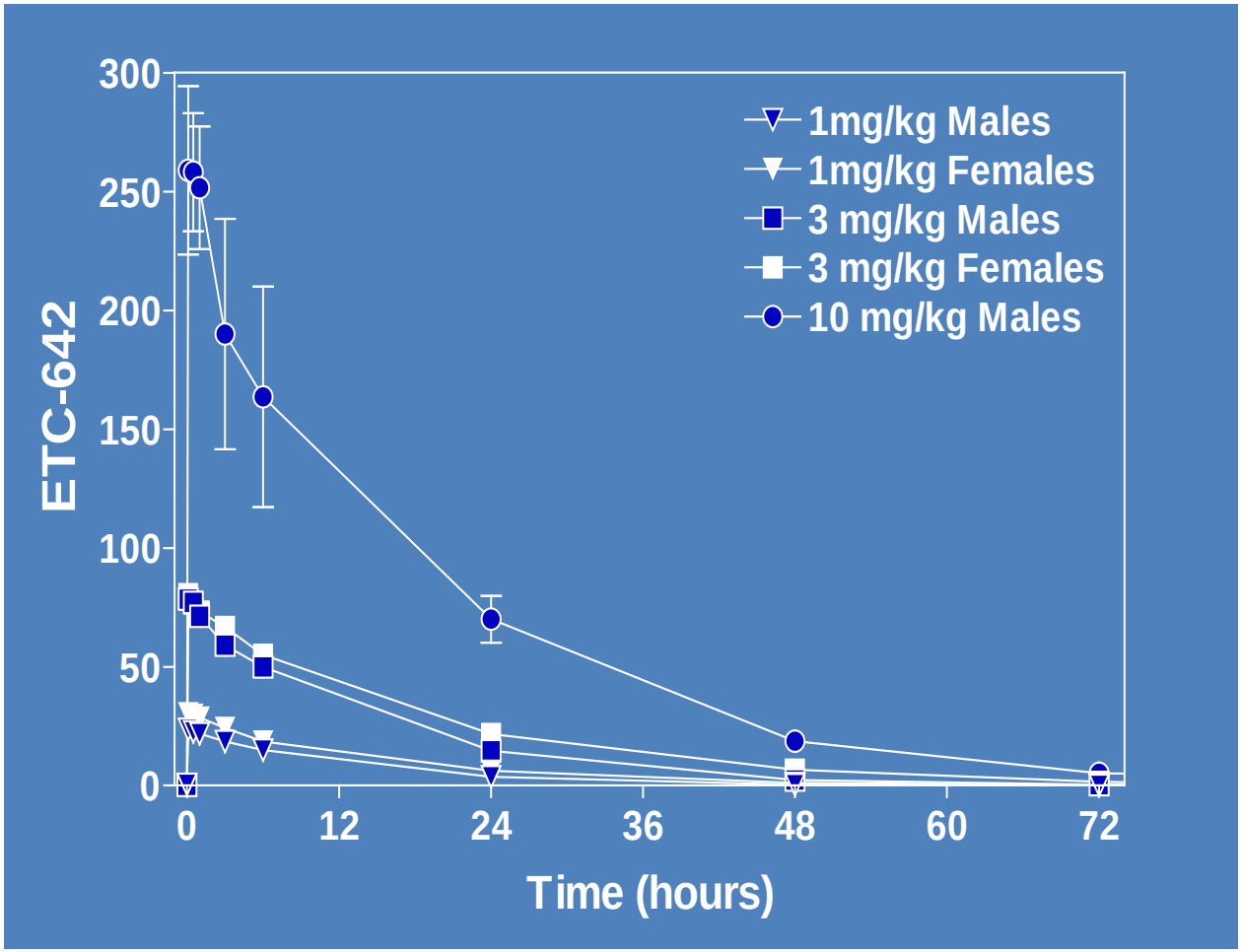


Parameter	HDL Level		p value
	< 20 mg/dL	≥ 20 mg/dL	
30 day mortality	16/30 (53%)	3/33 (9%)	<0.01
ICU stay >7 days	18/21 (86%)	17/39 (57%)	0.03
Acquired infection	10/21 (48%)	3/30 (10%)	<0.01

Preparation and Characterization of ETC-642

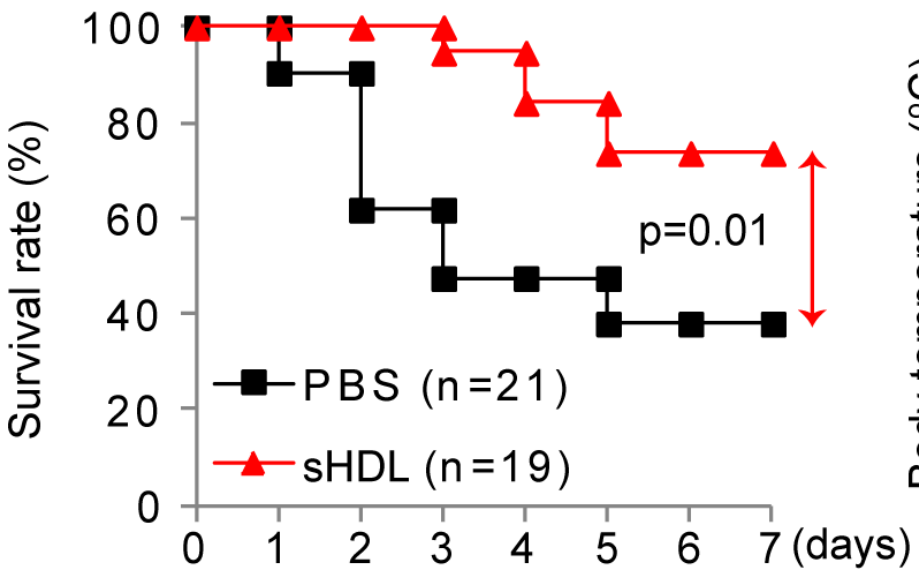


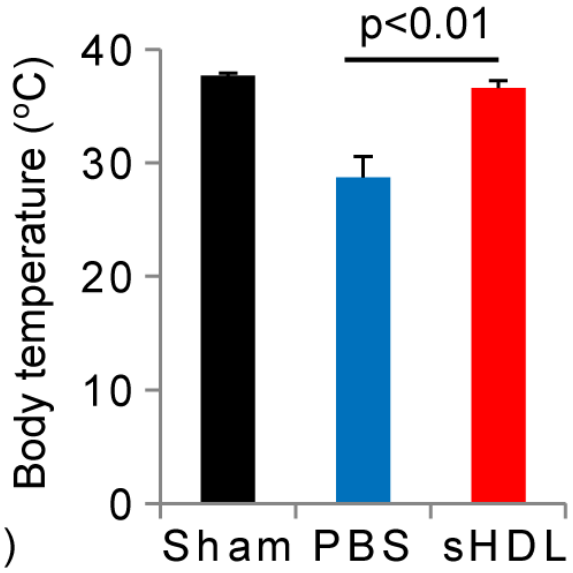
ETC-642 Pharmacokinetic Profile



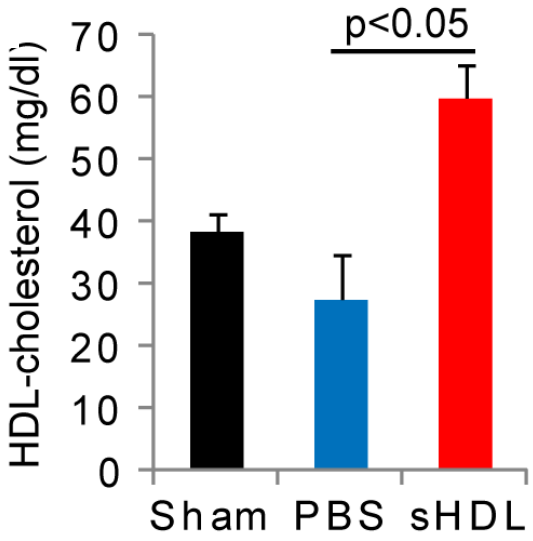
Dasseux, Proceedings of TIDES, 2004

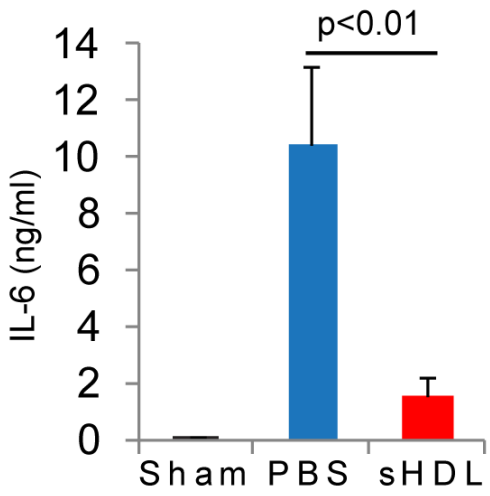
ETC-642 Infusion Rescues Septic Mice

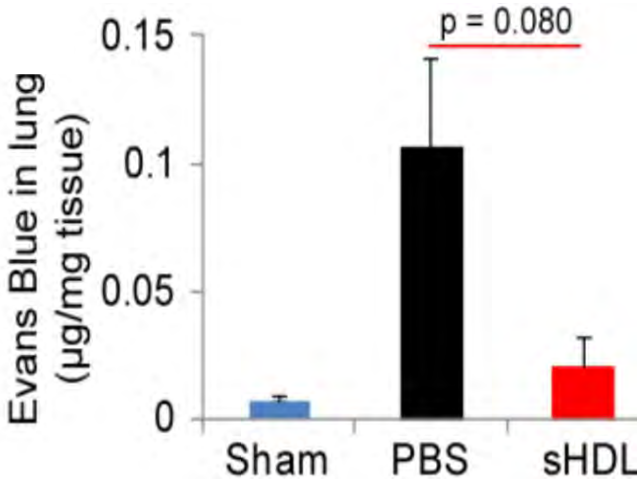




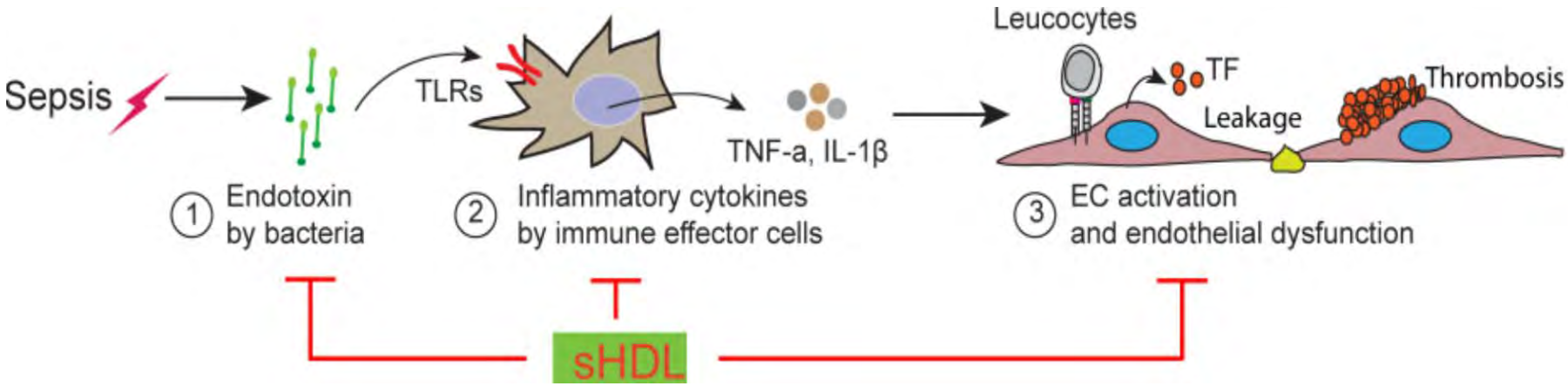
ETC-642 Restores HDL-C Levels, Prevent Cytokine Release and Endothelial Leakage



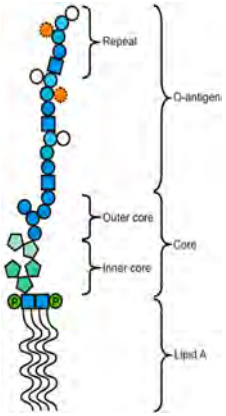


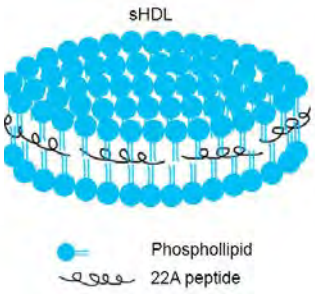


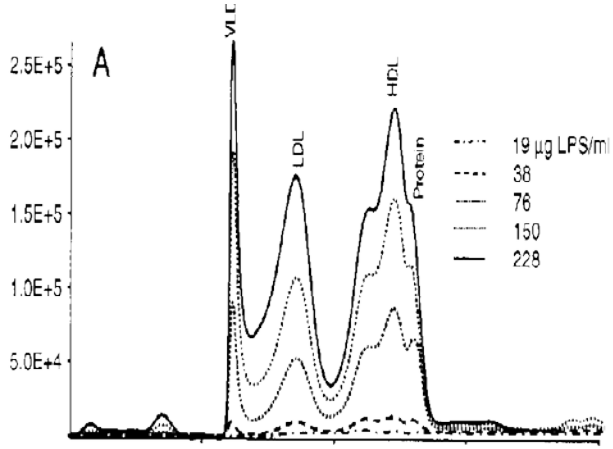
Mechanisms of HDL Protection in Sepsis



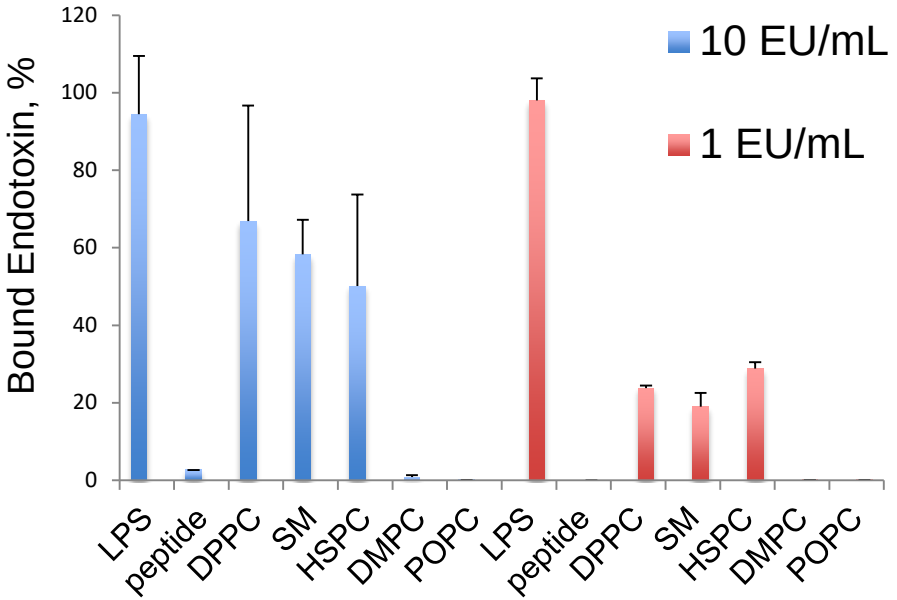
Lower Tg better physical binding of LPS





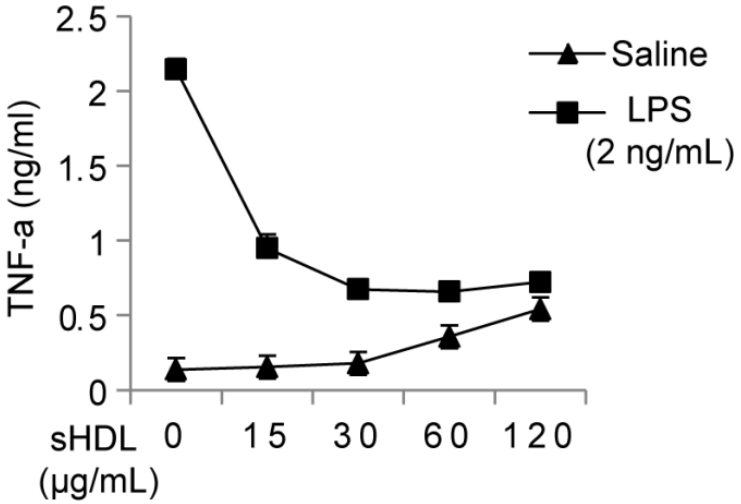


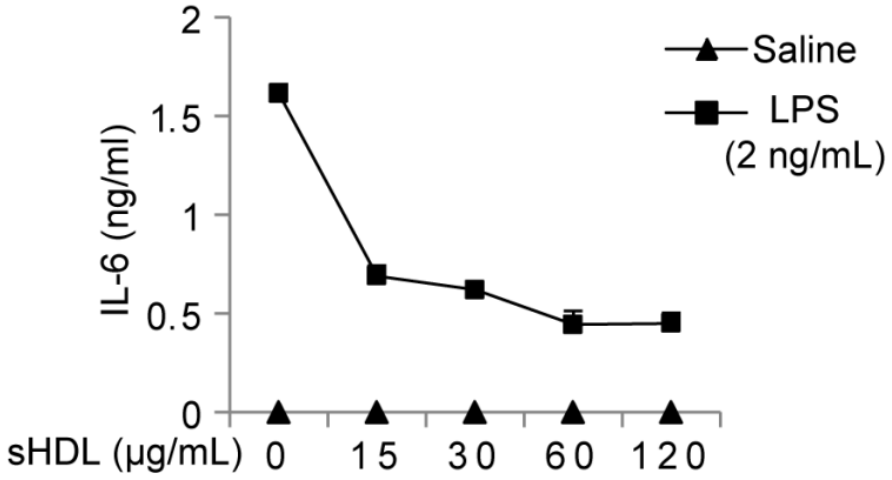
Levels, J. H., Infect Immun, 2001



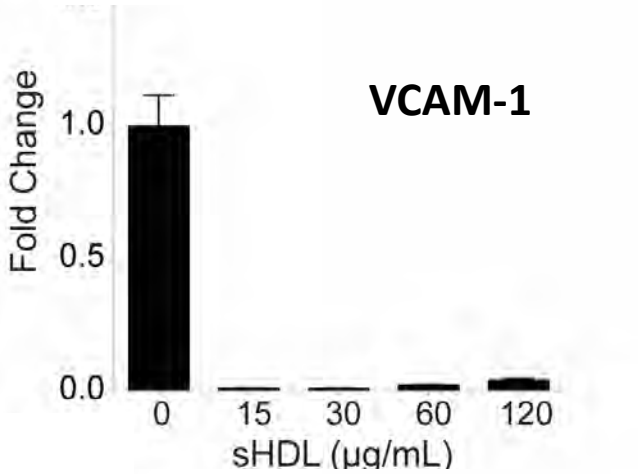
Emily Morin

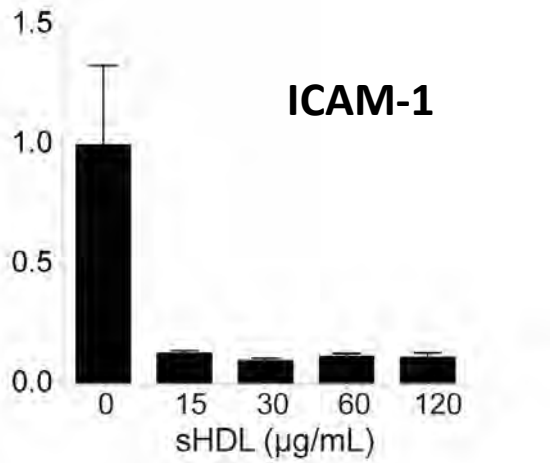
ETC-642 Inhibits Macrophage Cytokine Release

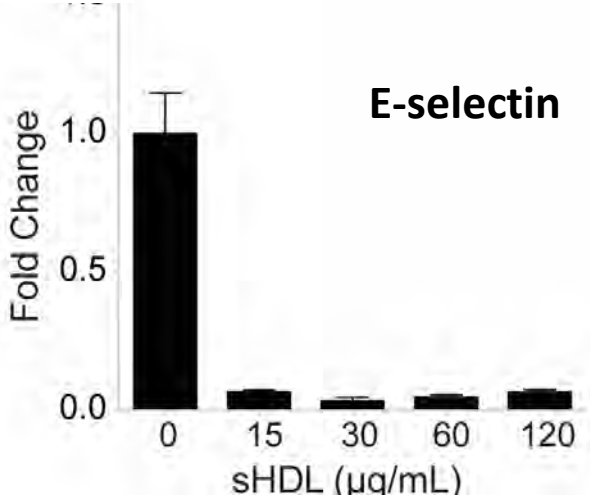


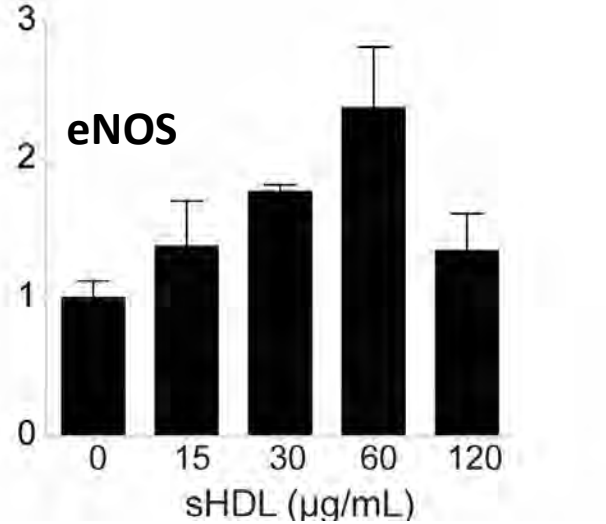


ETC-642 Inhibits Inhibits Endothelial Dysfunction









nature materials

ARTICLES

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Designer vaccine nanodiscs for personalized cancer immunotherapy

Rui Kuai[1,2], Lukasz J. Ochyl[1,2], Keith S. Bahjat[3], Anna Schwendeman[1,2*†] and James J. Moon[1,2,4*†]

HDL Vaccine Delivery Platform for Personalized Cancer Immunotherapy

James Moon, PhD

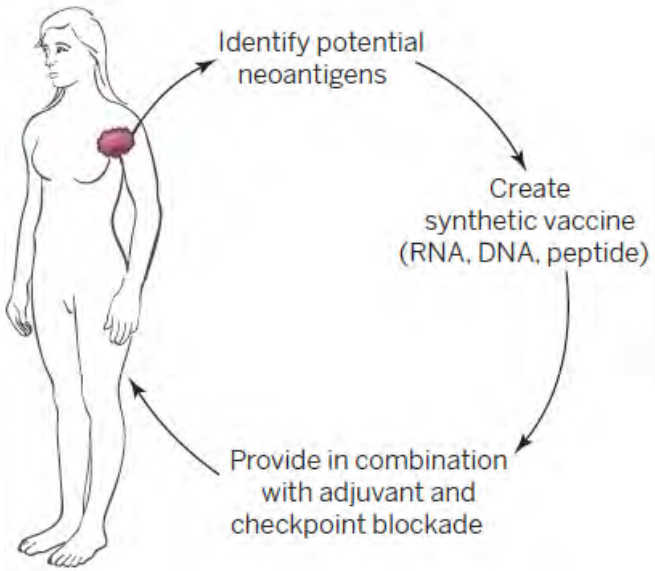
EVOQ THERAPEUTICS

Cancer immunotherapy has made significant progress, but major challenges remain

- Cancer immunotherapy has made international headlines with the striking clinical success of immune checkpoint inhibitors (e.g. Yervoy®, Keytruda® and Opdiva®)
    - However these approaches work by augmenting pre-existing anti-tumor immunity and only work in 20-40% of patients.
- Cancer vaccines elicit anti-tumor immunity *de novo* (regardless of pre-existing immunity), and hold promise as an alternative and complimentary approach to immunotherapy.
    - But, conventional cancer vaccines have failed to optimally deliver antigens and adjuvants to the draining lymph nodes and have demonstrated only limited efficacy
    - New cancer vaccines based on patient-specific neo-antigens, which have attracted strong investor and pharma interest, face the same delivery challenges

(Immunity. 2013; 39 (1): 1-10)

sHDL for personalized neo-antigen vaccination



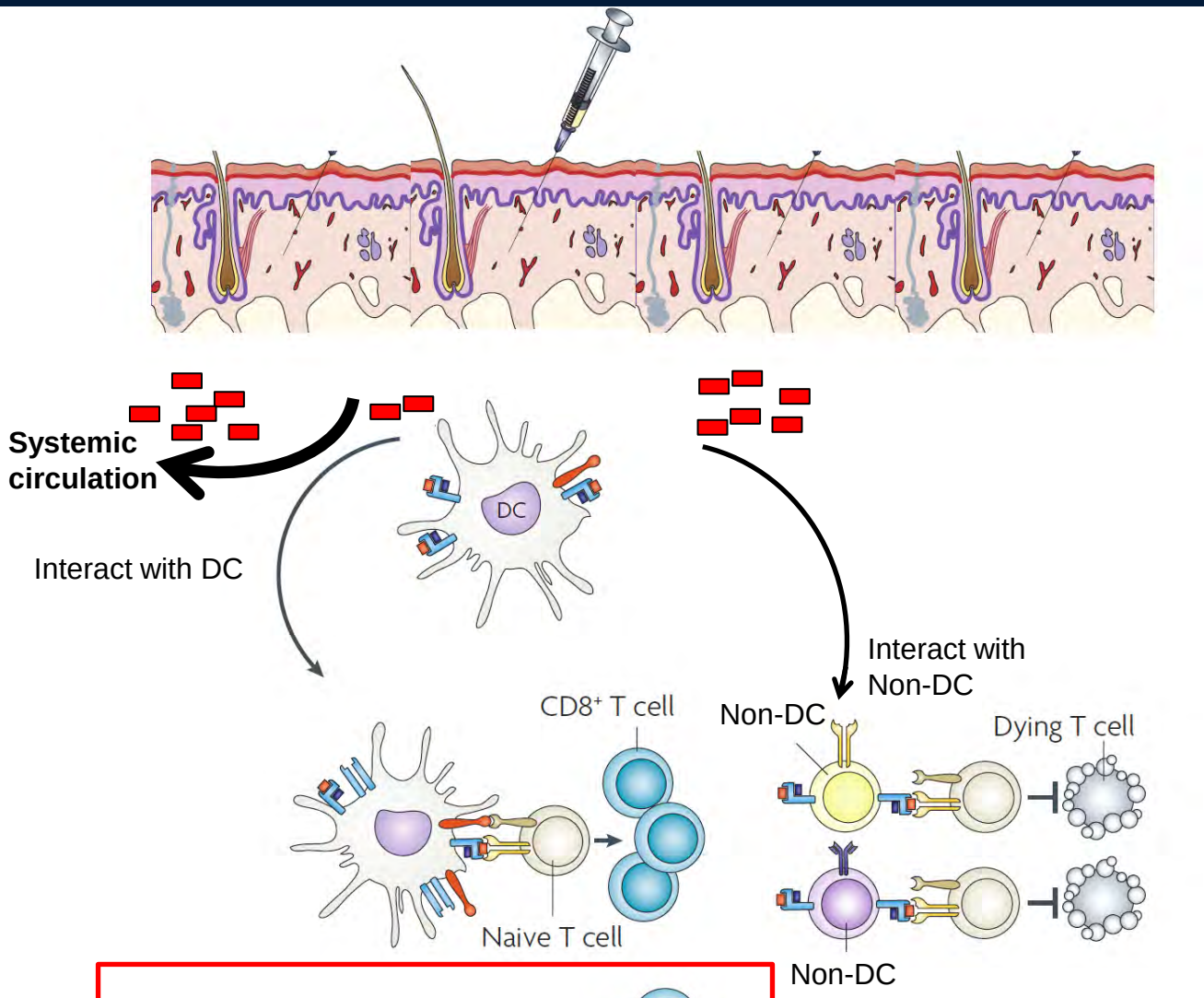
(Science. 2015; 348 (6230): 69-74)

Tumor cells

DNA/RNA sequencing

Neo-antigens

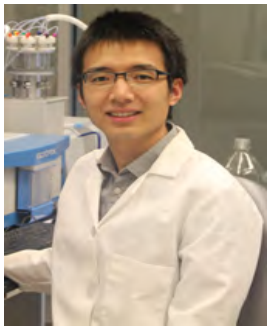
Limited efficacy of conventional peptide-based vaccines



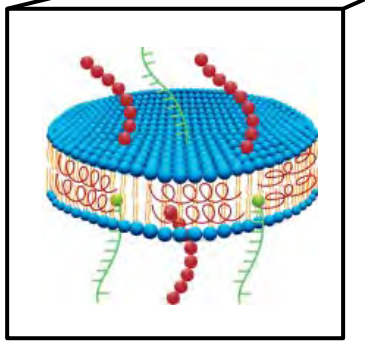
Weak T cell response

(Drug Discovery Today: Technologies 2015, 2(1), 89–96; Advanced Drug Delivery Reviews, 2011, 63(10-11), 890–900; Nat Rev Cancer. 2008;8(5):351-60.)

Research outline:
sHDL for personalized neo-antigen vaccination

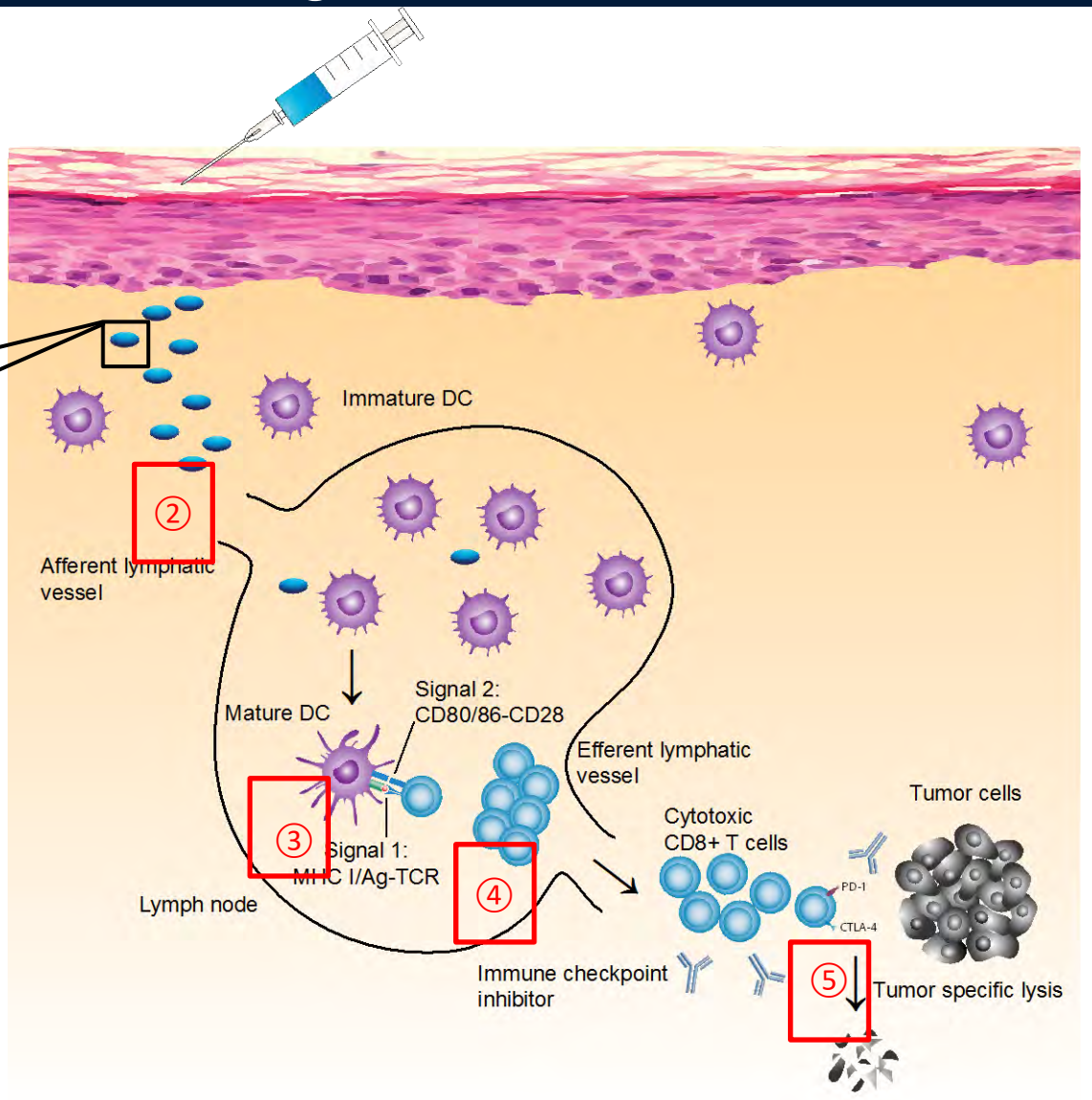


Rui Kuai



sHDL-Ag/CpG

①



① Formulation; ② Lymph nodes draining; ③ Antigen presentation; ④ CD8+ Cytotoxic T lymphocytes (CTLs) proliferation; ⑤ CTLs function

Synthetic HDL nanodiscs: an ideal platform for cancer vaccine delivery

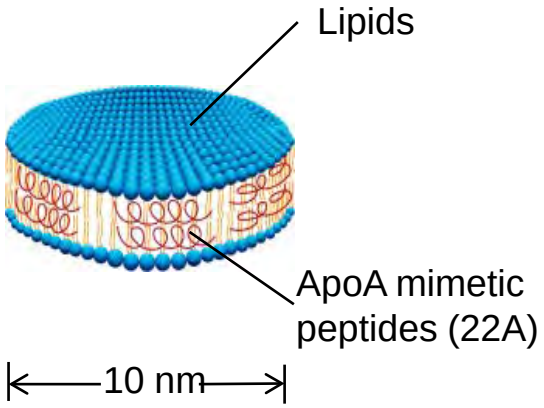
Synthetic HDL nanodiscs are an ideal platform for cancer vaccine delivery:

1. 10 nm size →
   direct access to lymph nodes

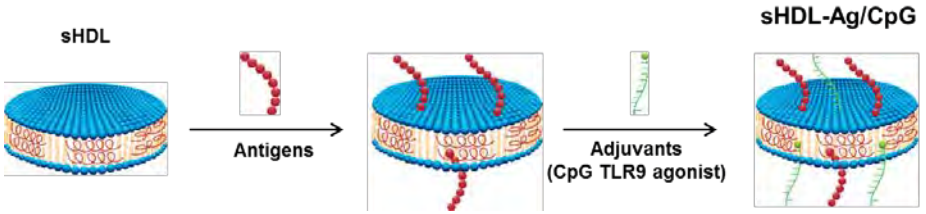
2. Multiple cargo loading sites →
   co-delivery of antigen and adjuvant

3. Clinical stage development of nanodiscs →
   safe, well-characterized, scalable manufacturing

Synthetic High Density Lipoprotein nanodisc (sHDL)

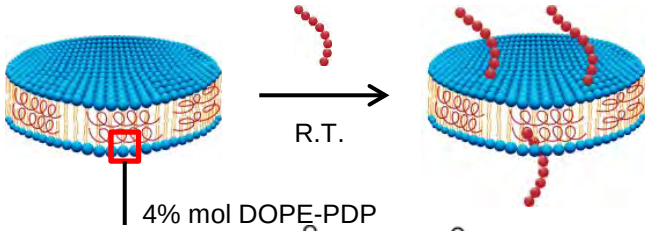


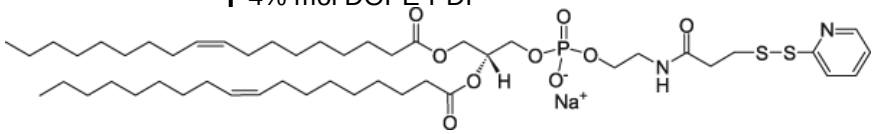
Conjugation of sHDL with antigens and adjuvants



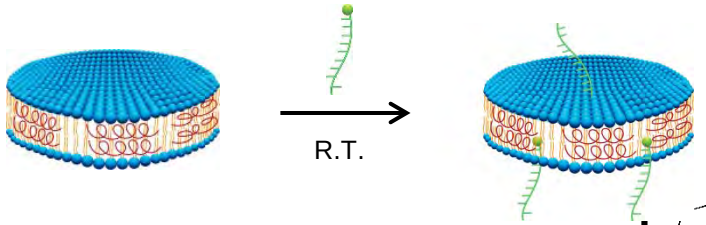
Loading Ag peptides and adjuvants to sHDL nanodiscs

CSS-Antigen



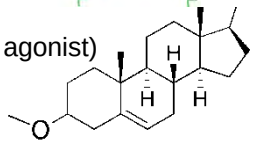


Cho-CpG (TLR9 agonist)

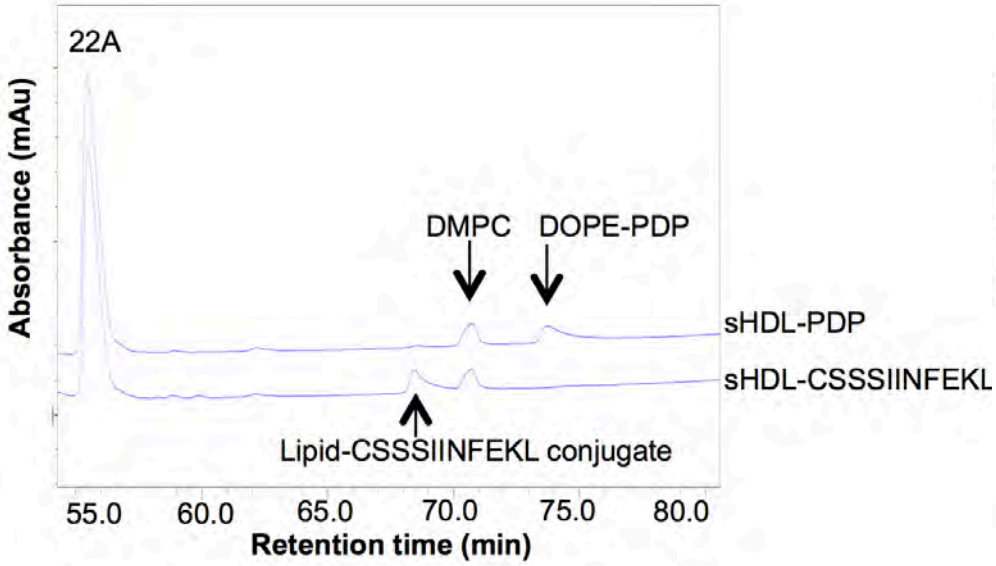


Cho-CpG (TLR9 agonist)

5’-tccatgacgttcctgacgtt-3’

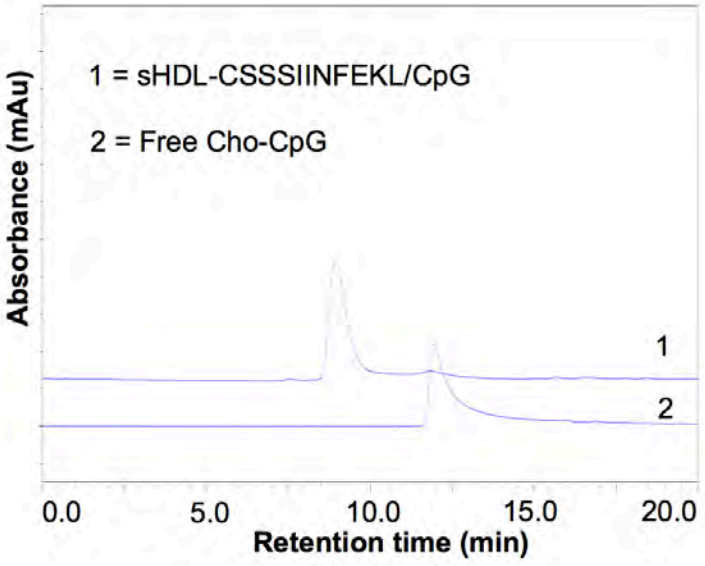


HPLC chromatograms



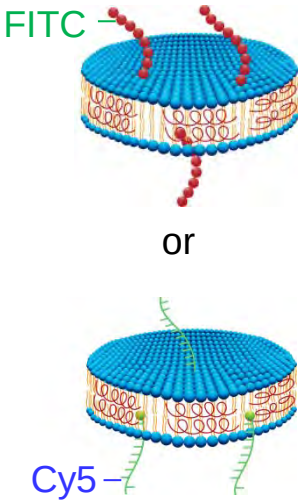
Loading efficiency > 90%
(7.5 ± 2.5 Ag per sHDL)

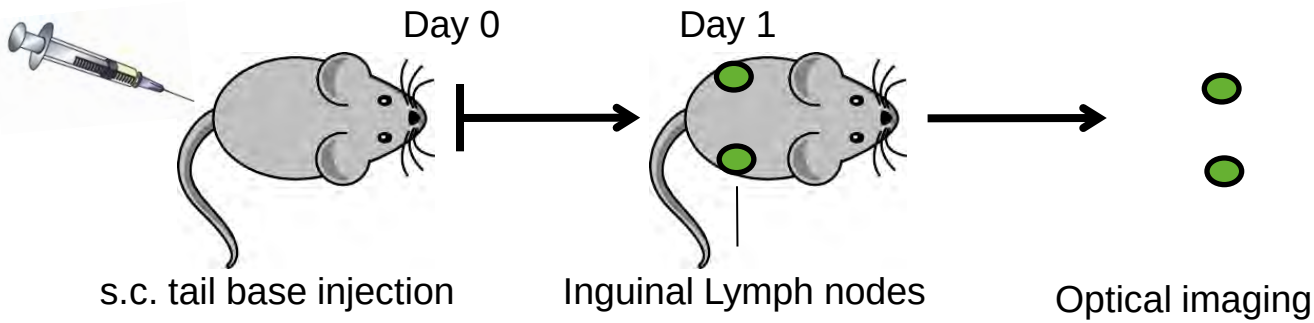
Gel Permeation Chromatography (GPC)



Loading efficiency > 98%
(1.2 ± 0.2 CpG per sHDL)

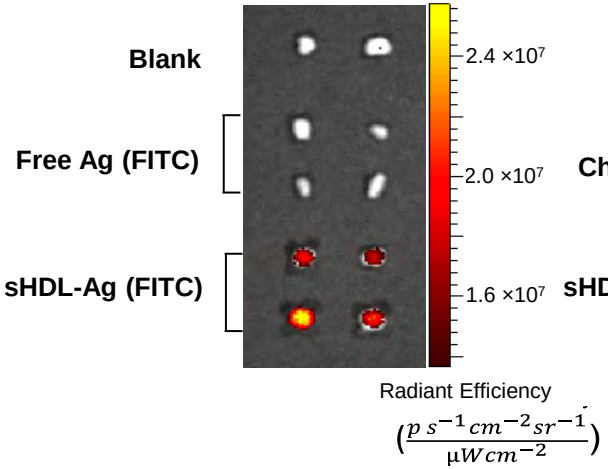
sHDL nanodiscs increase draining of antigens and adjuvants to lymph nodes

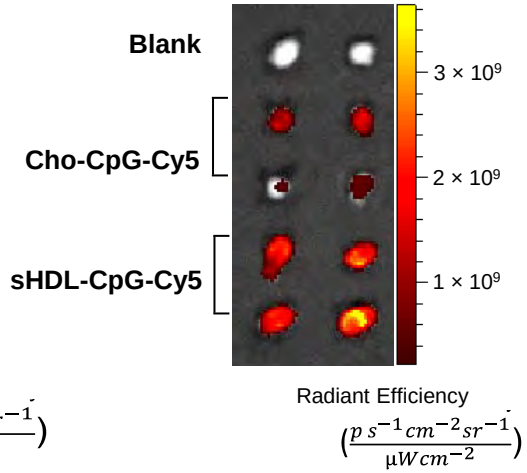


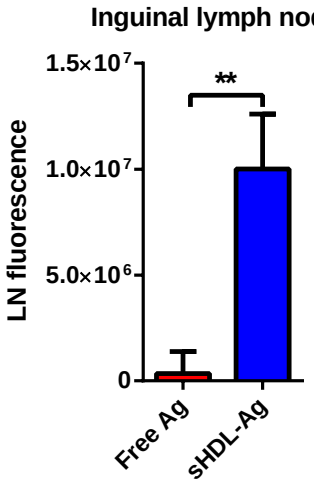


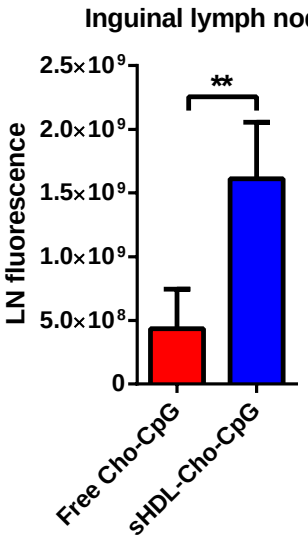
28-fold increase

3-fold increase



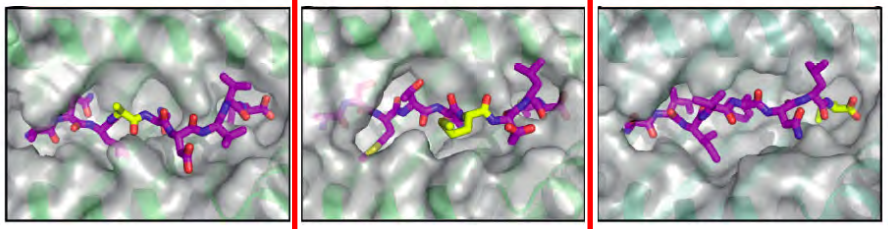




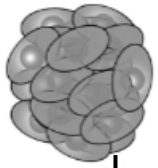


Sequencing neoantigen (Adpgk) in MC38 colon cancer cells

Protein	Reps1	Adpgk	Dpagt1
MHC haplotype	H-2D^b	H-2D^b	H-2K^b
Mutant	AQLANDVVL	ASMTNMELM	SIIVFNLL
Wild type	AQLPNDVVL	ASMTNRELM	SIIVFNLV
Solvent-exposed or anchor residue	Solvent	Solvent	Anchor



Nature. 2014;515(7528):572-6.

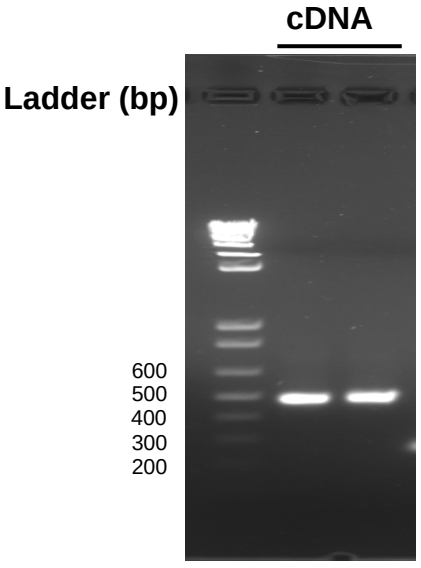


MC-38 tumor cells

Total RNA

cDNA

Neo-antigen cDNA



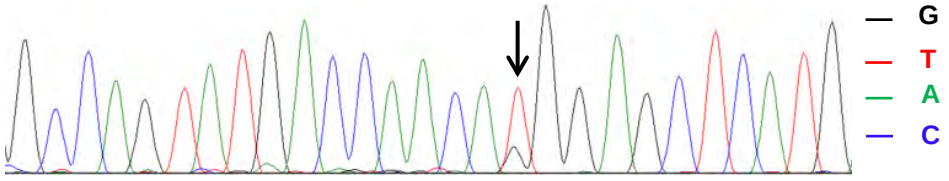
cDNA sequencing

WT: G C C A G T A T G A C C A A C A G G G A G C T C A T G

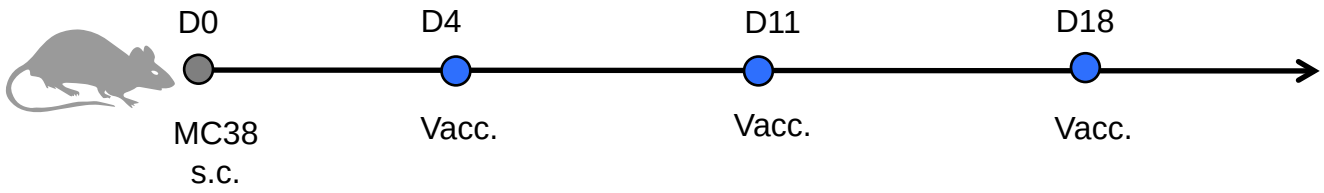
WT peptide: A S M T N R E L M

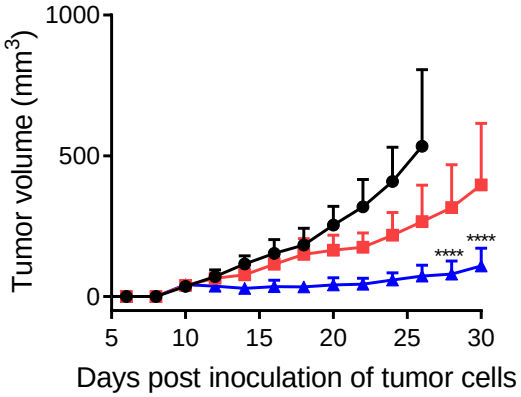
Mutant: G C C A G T A T G A C C A A C A T G G A G C T C A T G

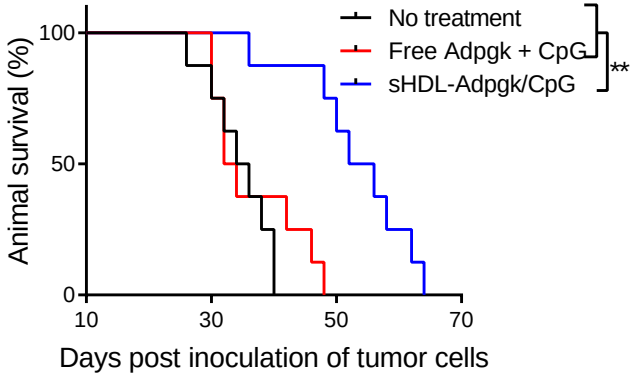
Mutant peptide: A S M T N M E L M

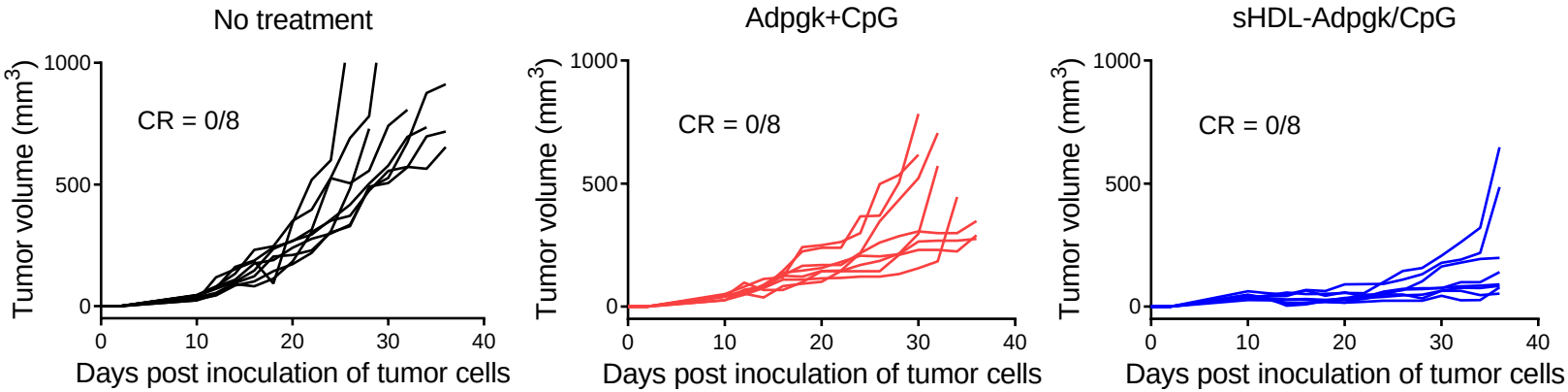


Neoantigen-specific CD8+ T cells delay tumor growth in a therapeutic setting



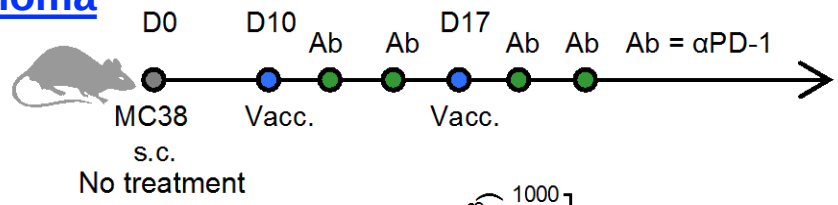




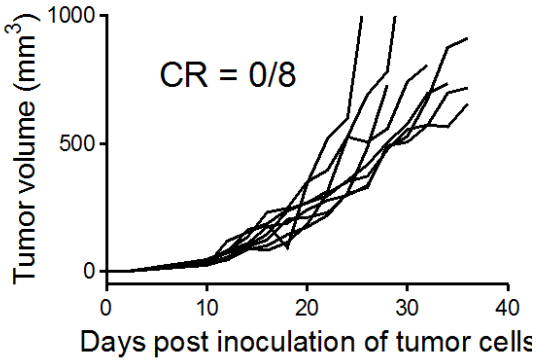


Elimination of tumors after nanodisc vaccination plus immune checkpoint blockade

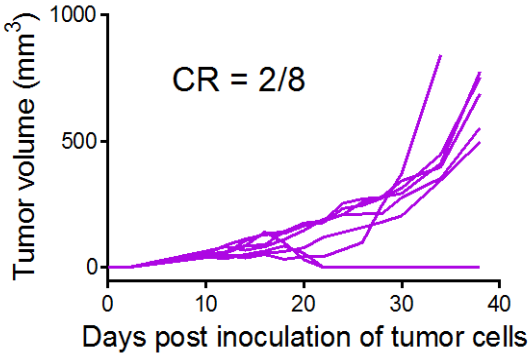
Colon carcinoma

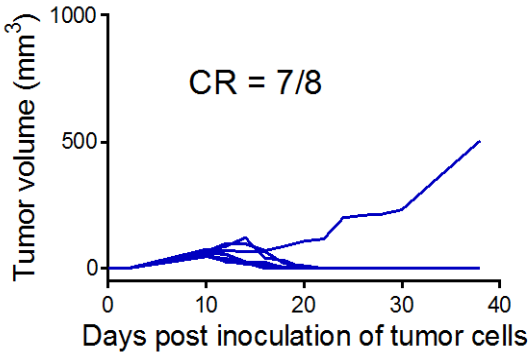


No treatment

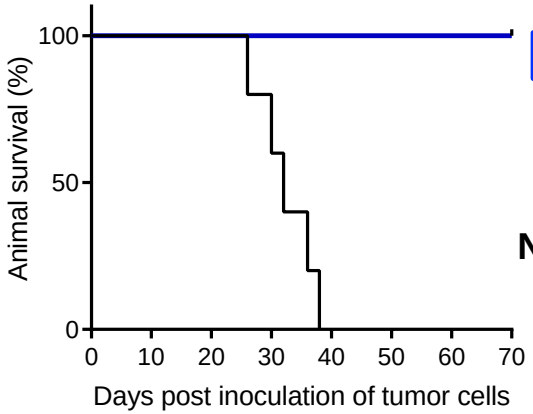


sHDL-Adpgk/CpG+αPD-1





Long-term protection against tumor recurrence



Nanodisc

No Treatment

MC38 re-challenge on day 70 by s.c. or i.v. route

Summary

- Despite positive early clinical trials there is still no approved sHDL products
- Design of easy to manufacture functional synthetic ApoA-I peptides could be an alternative to high cost protein
- More data is needed on what is the optimal lipid composition, dose, dosing intervals
- Novel sHDL appear to be useful for treatment of sepsis and potentially other diseases
- This “nature’s nanoparticle” could be modified to effectively deliver antigens and drugs

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