Targeted, Functional Lipid Nanoparticles as Cancer Therapy and Translation

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Department of Urology

Lab Focus: Synthesis of materials that mimic the form of naturally occurring nanostructures, but with novel function(s), to create next generation therapies.
Outline

• Historical Research Context
  – Functional therapeutic nanomaterials

• Current Research Focus
  – Functional high-density lipoprotein nanoparticles for lymphoma, CLL
  – High-density lipoprotein nanoparticles for nucleic acid delivery

• Future and Emerging Directions

• Commercialization
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Heart Disease

Problem:
- Heart disease is the number one cause of mortality in the world
- 720,000 heart attacks/ year
- 600,000 deaths (1 in 4 deaths in US)

Cause is Atherosclerosis:
- Hyperlipidemia: Cholesterol, LDL-C
- Vascular inflammation
- Turbulent blood flow

Cholesterol

Lipoproteins Are Natural Nanostructures That Transport Cholesterol

Braunwald's Heart Disease, 8th Ed.
HDL-C Levels Are Correlated with Reduced CHD Risk

Women

Men

![Graphs showing the correlation between HDL cholesterol levels and relative risk for CHD in men and women, based on data from Sharrett, Patsch, et al., Circulation 104:1108, 2001.](image_url)
Nanoparticle-Templated High Density Lipoprotein Nanoparticles (HDL NPs)

5 nm
(citrate)

APOAI

1

Phospholipids

2

Spherical Tailorabale Natural Function? New Function(s)?

Increasing HDL-mediated cellular cholesterol efflux is a promising therapeutic strategy for combating atherosclerosis. Functional mimics of HDL (fmHDL) can be made using a gold nanoparticle template that is functionalized with apolipoprotein A-I (apo A-I) and a phospholipid bilayer. Transmission electron microscopy confirms gold nanoparticle surface functionalization. The electron dense gold nanoparticles are visible as black spheres, and the apo A-I and phospholipids are seen as white rings around the black spheres. Studies reported in this issue show that fmHDL synthesized with apo A-I promote cellular cholesterol efflux through the same pathways used by native HDL species, including passive diffusion, ABCG1- and SR-BI-mediated diffusion, and ABCA1. (See Luthi et al., p. 972.)
HDL NPs are a High Affinity Ligand for Scavenger Receptor Type B-1 (SR-B1): Modulates Cholesterol Flux
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Patients with extremely low high-density lipoprotein-cholesterol (HDL-C) pose distinct challenges to clinical diagnosis and management. Confirmation of HDL-C levels below 20 mg/dl in the absence of severe hypertriglyceridemia should be followed by evaluation for secondary causes, such as androgen use, malignancy, and primary monogenic disorders, namely, apolipoprotein A-I mutations, Tangier disease, and lecithin-cholesterol acyltransferase deficiency. Global cardiovascular risk assessment is a critical component of comprehensive evaluation, although the association between extremely low HDL-C levels and atherosclerosis remains unclear. Therapeutic interventions address reversible causes of low HDL-C, multi-organ abnormalities that may accompany primary disorders and cardiovascular risk modification when appropriate. Uncommon encounters with patients exhibiting extremely low HDL-C provide an opportunity to directly observe the role of HDL metabolism in atherosclerosis and beyond the vascular system.

*J Clin Endocrinol Metab 97: 3399–3407, 2012*
SR-B1 is Over Expressed in Cancer

With Denise Scholtens, PhD and Young Chae Kwang, MD
B Cells and Diffuse Large B Cell Lymphoma (DLBCL, NHL)
Lymphoma Cells Overexpress SR-B1

Overexpressed SR-B1

Lymphoma Cell

Normal Lymphocyte

Human B Cell Lymphoma Samples (n=20/group) vs Normal B Cells (n=3/group)

Northwestern Medicine

With Dr. Amy Chadburn
Yang, et al. PNAS, 2013
HDL NPs Reduce Viability of SR-B1 Expressing Lymphoma Cells

Yang, et al. PNAS, 2013
Antibody Blockade of SR-B1 Prevents HDL NP Induced Cell Death

*\(p<0.05\)
HDL NPs Abolish Lymphoma (Ramos) Tumor Growth in Mouse Model (SCID Mouse/ Xenograft)

HDL NPs (1μM, 100μL) administered via tail vein, 5 daily doses over 11 days

Yang, et al. PNAS, 2013
Comparison of HDL NP vs Doxorubicin in SUDHL-4 Xenograft (SCID Mouse) : 3X/Week Dosing

HDL NP (1.0\mu M, 100\mu L) and doxorubicin administered via tail vein, 3X/week, over ~28 days
HDL NPs Are Not Toxic to Normal Human Lymphocytes Which Do Not Express SR-B1

Apoptosis (fold over control)

[Image showing bar graphs for HDL NP treatment and days post treatment]

Yang, et al. PNAS, 2013
HDL NP is Not Toxic To Normal, Primary Human Hepatocytes/ Macrophages

SR-B1

Hepatocytes  HepG2  Jurkat

GAPDH

Hepatocytes  HepG2

SR-B1/GAPDH  0.99  1  0.01

Macrophages  HepG2

0.81  1

Yang, et al. PNAS, 2013
B Cell Receptor Signaling and Targets
B Cells and B Cell Chronic Lymphocytic Leukemia

Collaboration:
Dr. Frank Giles
Dr. Paolo Ghia (Italy)
Dr. Cristina Scielzo (Italy)
Kaylin McMahon
Dr. Linda Foit
SR-B1 Receptor is Expressed in Patient CLL Cells

WB analysis on CLL patient samples:

SR-B1

B-actin
CLL Cells Apopotosis After 72h Treatment HDL NPs

30NM 72h

% cells CD19 CD5 PI+

Total PBMC

ns

30NM 72h

% cells CD19 CD5 PI+

Isolated B cells

**

100nM 72h

% cells CD19 CD5 PI+

Total PBMC

*

100nM 72h

% cells CD19 CD5 PI+

Isolated B cells

****

Total patients= 10

Total patients= 9

Total patients= 8

MBL = 1

Total patients= 6
CLL Specific Clone Cell Apopotosis After 72h Treatment with 100 nM HDL NPs

Populations 72h 100nM NP

%PI cells

CLL clone  B cells  T cells

*
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Opportunities for HDL NPs in Addition to Inherent Targeting and Function
Nucleic Acids (NA) as Therapies for Cancer

**RNA Interference (RNAi):** Endogenous post translational RNA regulatory mechanism

**Benefits of NA Therapies:** Potent form of therapy and target/patient specific

**Challenges of Systemic RNAi Therapy:**

1. Short circulating half-life
2. Lack tissue penetration
3. Degradation limits efficacy
4. Lack of cell specific targeting
5. Off target effects
6. Cellular or carrier sequestration
7. Reticulo-endothelial system mediated clearance

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*References:*
- Vaishnaw et al. *Silence.*; Vol 1, 2006
SR-B1 is Overexpressed in Numerous Human Cancer Types

With Denise Scholtens, PhD and Young Chae Kwang, MD
Molecular Mechanisms that Drive Castrate Resistant PCa and Current Therapy

Prostate Cancer Cell
Systemic Androgen Ablation: Leuprolelin, Goserelin

Finasteride
Dutasteride

Abiraterone

Statins

Flutamide

17-AAG
MDV3100
HDL

HMGCR
Acetyl-CoA

Cholesterol
Testosterone

5α-reductase

CYP17A1

Coactivator

Survival
Cell Growth
Proliferation

Heat-shock Protein

Coactivator

ARE

AR

DHT

Systemic Androgen Ablation:

Leuprolelin
Goserelin

Molecular Mechanisms that Drive Castrate Resistant PCa and Current Therapy

Scavenger Receptor Type B-1 (SR-B1) is Expressed in PCa Tissue

Human Tissue Microarrays:

- Normal
- PIN
- Gleason 3 + 3
- Gleason 4 + 4
- Gleason 4 + 5
- LN Met

Samples were obtained from Don Vander Griend, PhD, University of Chicago With Steve Rohan, MD, Northwestern
Scavenger Receptor Type B-1 (SR-B1) is Expressed in PCa Cells

PCa Cell Lines:

<table>
<thead>
<tr>
<th>SR-B1</th>
<th>AR/N20</th>
<th>GAPDH</th>
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<tbody>
<tr>
<td>HepG2</td>
<td>THP-1</td>
<td></td>
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<tr>
<td>957e/hTERT</td>
<td>PriE</td>
<td></td>
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<tr>
<td>DU145</td>
<td>PC3</td>
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<tr>
<td>LNCaP</td>
<td>C42-B</td>
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<tr>
<td>LAPC4</td>
<td>CWR-22Rv1</td>
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<td>CWR-R1</td>
<td>VCAP</td>
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With Don Vander Griend PhD., U of C
Rationale for Targeting the Androgen Receptor Using Therapeutic siRNA

Full Length AR

AR Variant(s)

- AR amplification/overexpression
- Gain-of-function AR mutations
- Intracrine androgen production
- Overexpression of AR cofactors
- Ligand independent AR activation
- Constitutively active AR variants
siRNA HDL Nanoparticle Synthesis: Modular for Individual Patients

Step 1: HDL Nanoparticle Synthesis

AuNP (5 nm) → ApoAl → Phospholipids
Citrate Stabilized

Step 2: HDL NP + siRNA/DOTAP

DOTAP + siRNA → Vortex/Sonicate → Cholesterol = siRNA
**In vitro Efficacy:** siRNA-HDL NPs Reduce AR Expression in LNCaP Cells

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Lipo AR</th>
<th>Lipo Ctrl</th>
<th>HDL NP 20 nM</th>
<th>HDL NP 10 nM</th>
<th>HDL NP 5 nM</th>
<th>siAR HDL NP 20 nM</th>
<th>siAR HDL NP 10 nM</th>
<th>siAR HDL NP 5 nM</th>
<th>siCtrl HDL NP 20 nM</th>
<th>siCtrl HDL NP 10 nM</th>
<th>siCtrl HDL NP 5 nM</th>
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<tbody>
<tr>
<td><strong>AR</strong></td>
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<tr>
<td><strong>B-actin</strong></td>
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</tbody>
</table>

24 hours

48 hours
In vitro Efficacy: siRNA HDL NPs Reduce AR Expression in LNCaP Cells

72 hours

96 hours

Untreated Lipo AR Lipo Ctrl HDL NP 20 nM HDL NP 10 nM HDL NP 5 nM siAR HDL NP 20 nM siAR HDL NP 10 nM siAR HDL NP 5 nM siCtrl HDL NP 20 nM siCtrl HDL NP 10 nM siCtrl HDL NP 5 nM
siRNA-HDL NPs Reduce LnCaP Cell Viability

- **24 Hours**
- **48 Hours**
- **72 Hours**
- **96 Hours**
AR-siRNA HDL NPs Regulate Target Gene Expression *In Vivo*

**Graph:**
- PBS
- Ctrl HDL NP
- AR HDL NP

**Western Blot:**
- Separated proteins

**Relative Protein Expression (AR/Tubulin):**
- PBS
- Ctrl HDL NP
- AR HDL NP

**Diagram:**
- siRNA HDL NP (100 μL, 1μM)
- 48 Hours Animal Sacrifice
- Harvest Tissue
- With Olga Volpert, PhD and Elena Vinokour
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SR-B1 is Overexpressed in Numerous Human Cancer Types

With Denise Scholtens, PhD and Young Chae Kwang, MD
GFP-SR-B1 in A375 Melanoma Cells
HDL NPs Inhibit Exosome Uptake in A375 Melanoma Cells

A375 melanoma cells: 25 nM HDL NP, 1 μg/mL exosomes
Inhibiting Microenvironment and Distant Exosome Signaling via HDL NPs

- Exosome uptake occurs through lipid rafts
- HDL NPs bind to SR-B1 in lipid rafts leading to a clustering of SR-B1s and lipid raft dysfunction inhibiting cellular exosome uptake
Acknowledgements

**Atherosclerosis**
Dan Rader, MD  
George Rothblat, PhD  
Nick Lyssenko, PhD

**Lymphoma**
Leo Gordon, MD  
Amar Singh, PhD  
Shuo Yang, PhD  
Marina Damiano, PhD

**Angiogenesis/ Prostate**
Olga Volpert, PhD  
Don Vander Griend, PhD  
Steve Rohan, MD  
Chad Mirkin, PhD  
Dorina Veliceasa, PhD  
Elena Vinokour, PhD

**Tumor Biology**
Andy Mazar, PhD  
Andrey Ugolkov, PhD  
Lisa Hurley  
Irawati Kandela
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Opportunities for HDL NPs in Addition to Inherent Targeting and Function: The Intellectual Property (IP)
Teamwork Required!

Shad Thaxton, MD, PhD
- Asst. Professor of Urology at Northwestern University
- Founder: AuraSense, LLC, AuraSense Therapeutics
- MIT Technology Review's TR35 award
- Chairman, founding board member

Leo Gordon, MD
- Professor, Oncology Research, Northwestern University
- Co-Director, Hematologic Malignancy Program

Chad Mirkin, PhD
- Director of International Institute for Nanotechnology
- Professor, Northwestern University
- Founder: Nanosphere, AuraSense, LLC, AuraSense Therapeutics
- Member of President’s Council of Advisors on Science &Technology

Key Advisors
Frank Giles, MD
- Director Northwestern University Developmental Therapeutics Institute
- Significant clinical trial & industry expertise

Andy Mazar, PhD
- NU Entrepreneur in Residence, Director Center for Developmental Therapeutics
- Key bench to clinic & industry expertise

Ann Lefevre
- Technical Director, NU GMP Facility, industry expertise

Key Operating Team Members
Philip Bligh, Executive Director
- Led class IV medical device company through FDA PMA & GMP approval
- Founded & IPO’d Hi-tech start-up
- Former E&Y entrepreneur of the year

Technical Team
Kaylin McMahon, Linda Foit, PhD
AuraSense has Granted Teleios Exclusive License to the HDL Nanoparticle Technology, Including Issued Composition of Matter Patents in the US, Japan and Mexico, as well as all Related Pending Patents Elsewhere

- Company Operates With a Capital Efficient Model Utilizing Northwestern University Clinical & Scientific Development Resources
- Initial Target is HDL NP as Treatment for Relapsed/Refractory DLBCL
- Continued Pipeline Development and R&D Efforts
  - Burkitt’s Lymphoma, Follicular Lymphoma
  - Glioblastoma, Breast Cancer, Prostate Cancer
  - Drug Delivery (e.g. vincristine, Doxirubicin)
  - Targeted Immunomodulation (Melanoma, Sepsis)
  - Targeted Disruption/Manipulation of Exosome Biology (Anti-Metastatic, Ex-Vivo Targeted Drug Formulations)
Timeline and Capital Needs

Pathway

2014 2015 2016 ~ 2020

Animal Tox. IND Phase I Phase II Phase III

$4-6M* $4-5M ~$12-15M

Currently Seeking Seed Capital

Use of Funds

• Animal Toxicity, including pharmacokinetics and core animal toxicity
• Key cGMP manufacturing and scale-up capabilities
• IND filing & Approval
• Targeted exploration of additional indications for molecule
• Operational costs, including; Management, technical team, legal & finance, IP protection
• Key future hires include targeted clinical development, quality assurance & regulatory personnel
Questions?