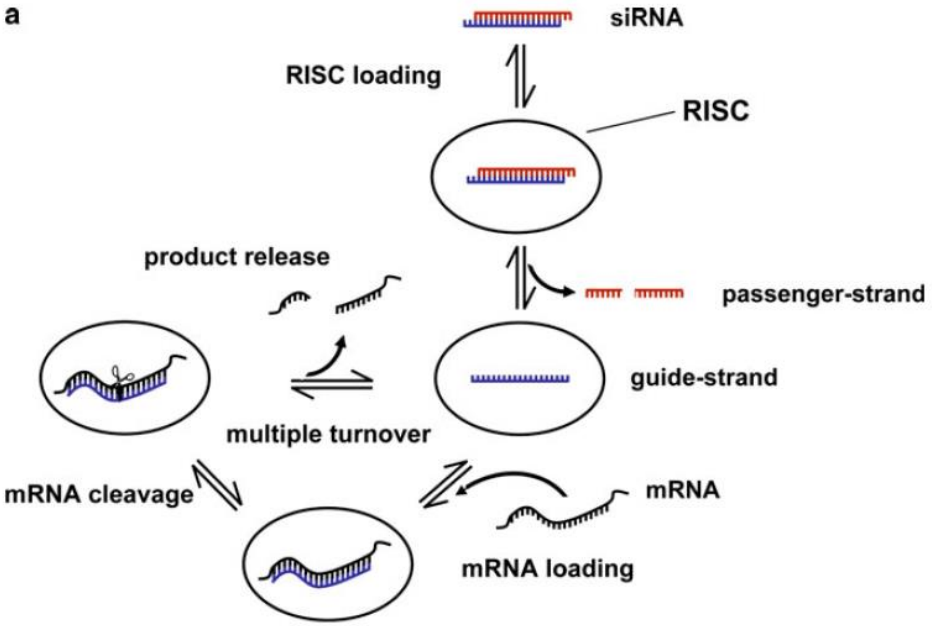
siRNA delivery targeting specific sites *in vivo*

Yvette Zarb

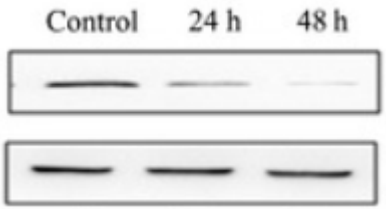
JC20161122

siRNA

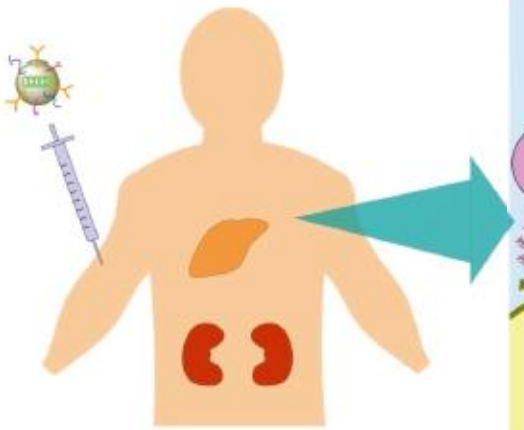
- Highly conserved biological process
- siRNA guides an RNA-inducing silencing protein complex
- Degrading the mRNA with the matching sequence

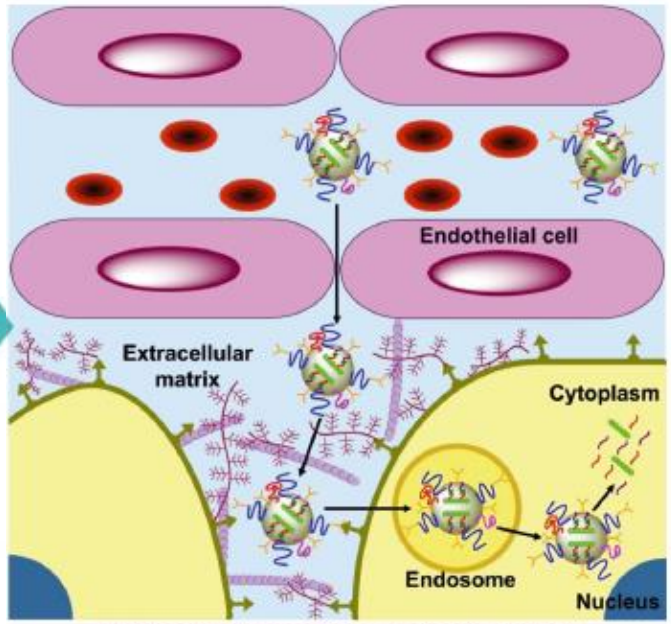


Laufer, Detzer, Sezakiel & Restle, 2010. RNA Technologies and their Applications



Challenges in RNAi *in vivo*





Circulation and biodistribution

Challenge:
- Off-target RNAi
- Degradation by nucleases
- Clearance by RES and renal filtration
- Nonspecific accumulation
- Unwanted systemic effects (e.g., toxicity)

Approach:
- Identification of most active siRNA sequence
- Chemical siRNA modification
- siRNA encapsulation
- Use of biocompatible and biodegradable carrier
- PEGylated carrier
- Tethered carrier with a targeting moiety
- Optimized administration route

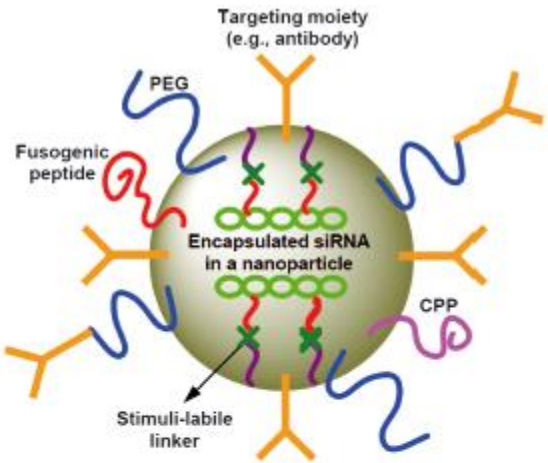
Extracellular and intracellular transport

Challenge:
- Endothelium penetration (extravasation)
- Diffusion through ECM network
- Specific binding to a target cell
- Cellular uptake (internalization)
- Endosomal escape (cytosolic release)
- siRNA dissociation from its carrier

Approach:
- Use of leaky vasculature in a certain type of tissue (e.g., tumor)
- Receptor-mediated specific binding to a target cell
- CPP-mediated internalization
- Use of fusogenic and endosome-destabilizing peptides/polymers
- Stimuli-cleavable polymers for siRNA release in the cytoplasm

Shim & Kwon, 2010. *FEBS Journal*

Challenges in RNAi *in vivo*



Component	Design goal
• siRNA complexed in a cationic polymeric nanoparticle	• Protection from enzymatic degradation; efficient transport and cellular uptake
• Polyethylene glycol (PEG) shell	• Retarded clearance by RES and renal filtration; reduced toxicity
• Targeting moiety (e.g., folic acid, peptide, or antibody)	• Specific binding to target tissue/cell
• Cell-penetrating peptide (CPP)	• Enhanced cellular internalization
• Fusogenic peptide or lipid, or endosome destabilizing polymer	• Facilitated siRNA release from the endosome
• Stimuli-labile linker	• Efficient siRNA dissociation from its carrier in the cytoplasm

Shim & Kwon, 2010. *FEBS Journal*

TECHNICAL REPORTS

nature medicine

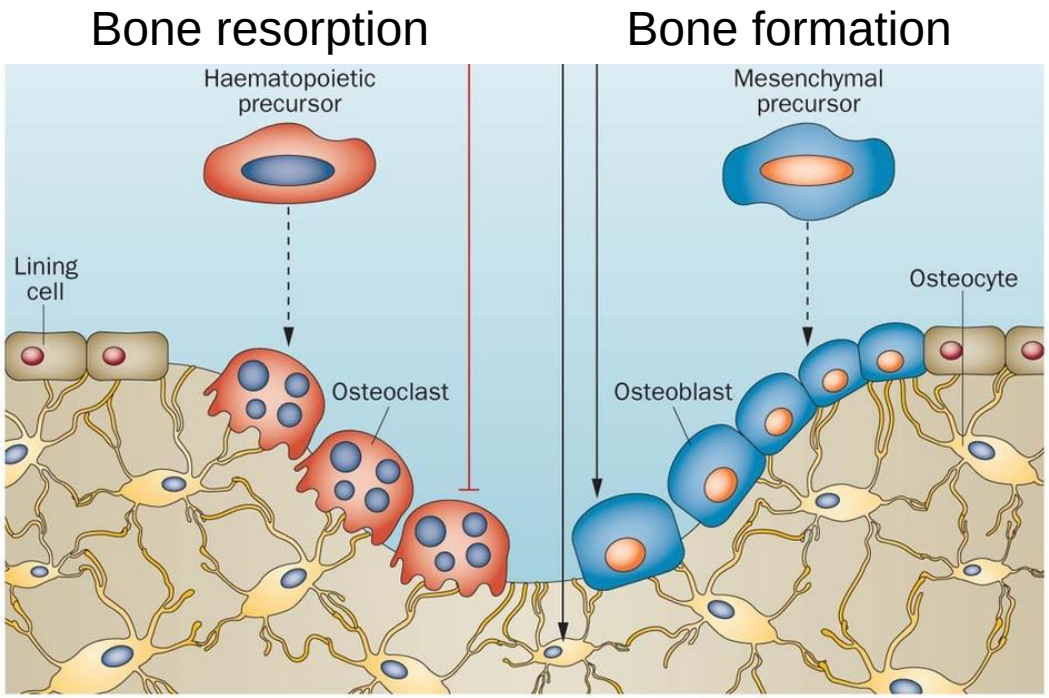
A delivery system targeting bone formation surfaces to facilitate RNAi-based anabolic therapy

Ge Zhang[1,13], Baosheng Guo[1,13], Heng Wu[1,13], Tao Tang[1,13], Bao-Ting Zhang[1,2,13], Lizhen Zheng[1], Yixin He[1], Zhijun Yang[3], Xiaohua Pan[4], Heelum Chow[5], Kinwah To[5], Yaping Li[6], Dahu Li[7], Xinluan Wang[1], Yixiang Wang[8], Kwongman Lee[9], Zhibo Hou[10], Nan Dong[11], Gang Li[1], Kwoksui Leung[1], Leungkim Hung[1], Fuchu He[7], Lingqiang Zhang[7] & Ling Qin[1,12]

Physical chemistry of bone targeting molecules

Oligopeptides

Bisphosphonates



?

Adapted from Manolagas, O'Brien & Almeida, 2013. *Nature review endocrinology*

Physical chemistry of bone targeting molecules

$(AspSerSer)_6$

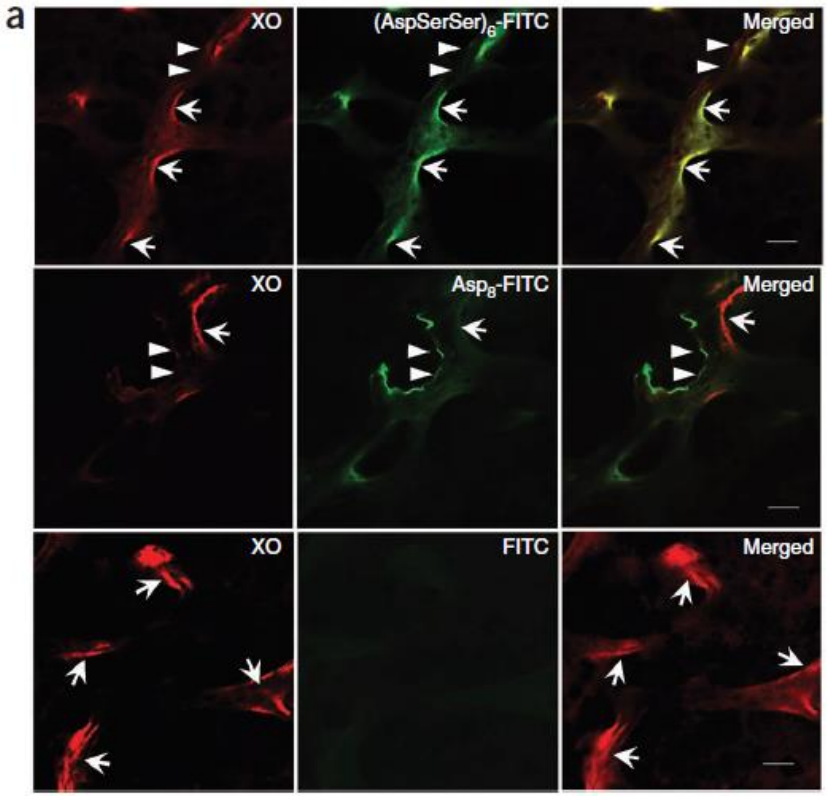
- Oligopeptide
- potential to target bone-formation surfaces
- Preferentially binds to lowly crystallized hydroxyapatite and amorphous calcium phosphonate

(Asp_8)

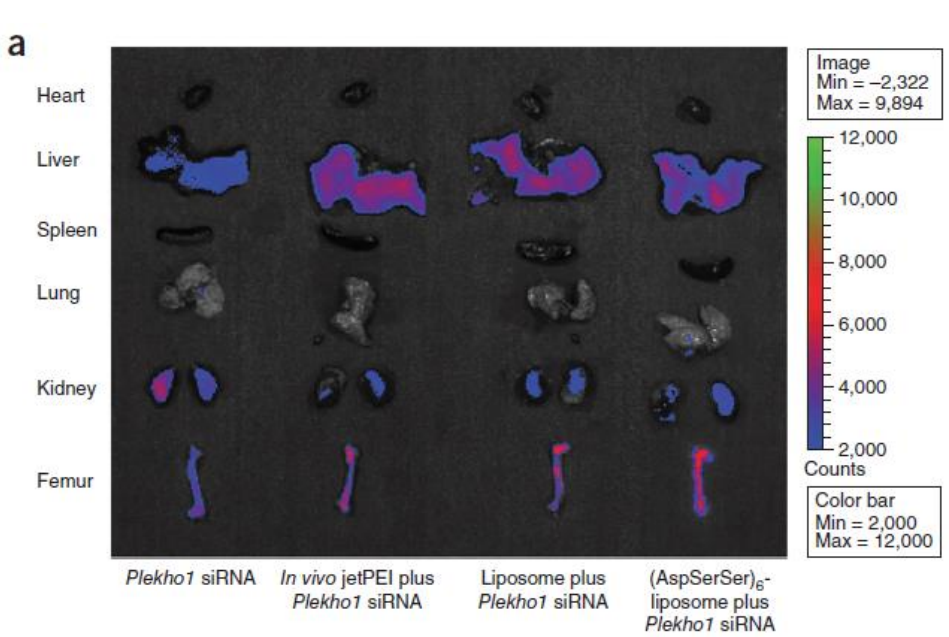
- Oligopeptide
- preferentially bind to bone-resorption surfaces
- stronger affinity to highly crystallized hydroxyapatite

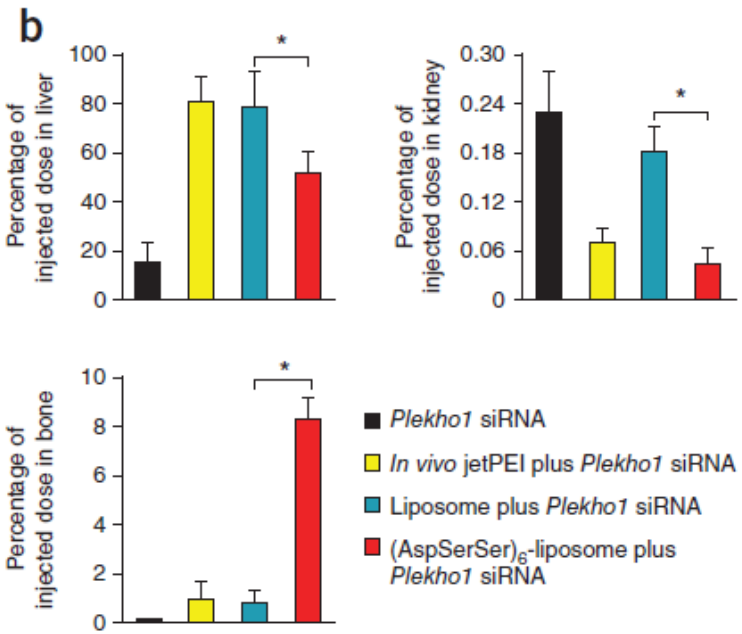
Differential occupancy characteristics of moieties at bone-formation or -resorption surfaces

- Xylenol orange marks bone formation surfaces
- $(AspSerSer)_6$ targets bone formation surfaces
- (Asp_8) target bone resorption surfaces

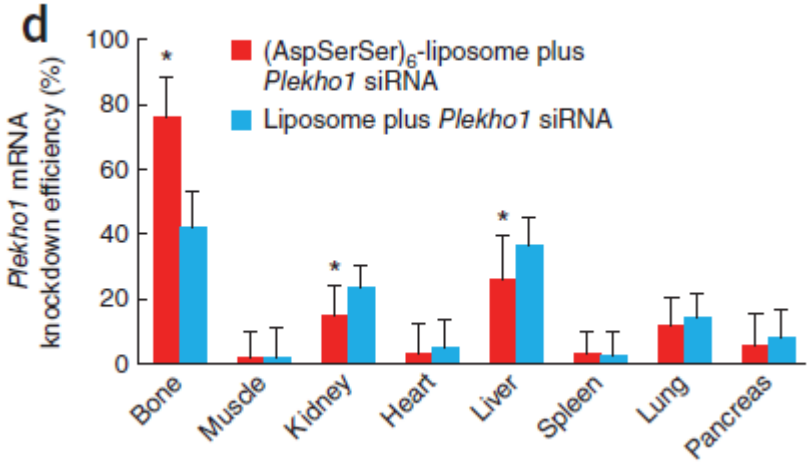


Characterization of the targeted delivery system *in vivo*

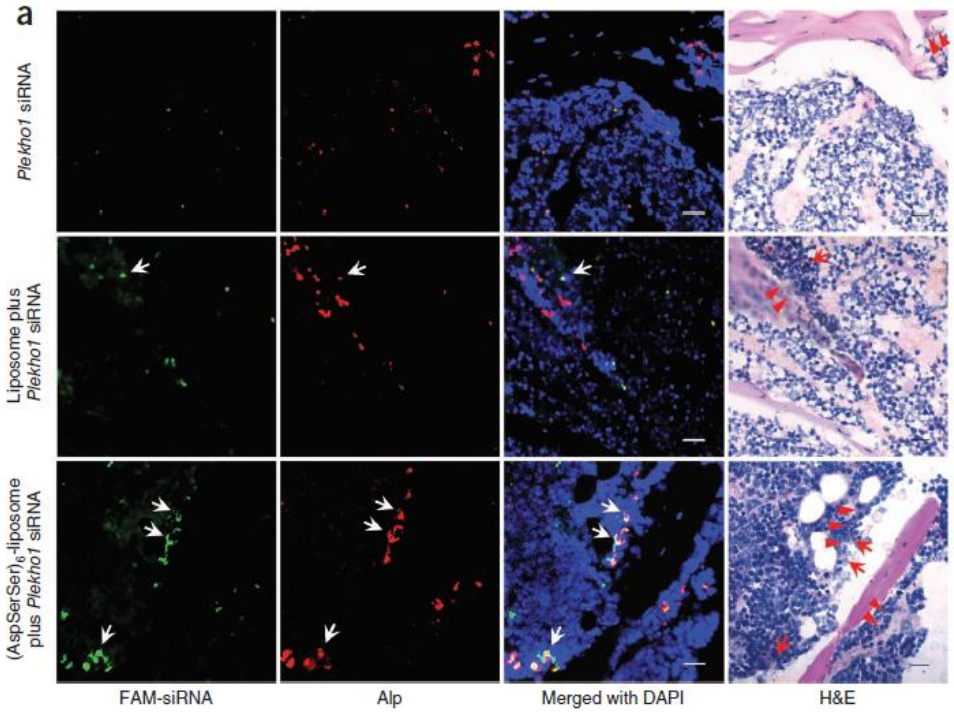


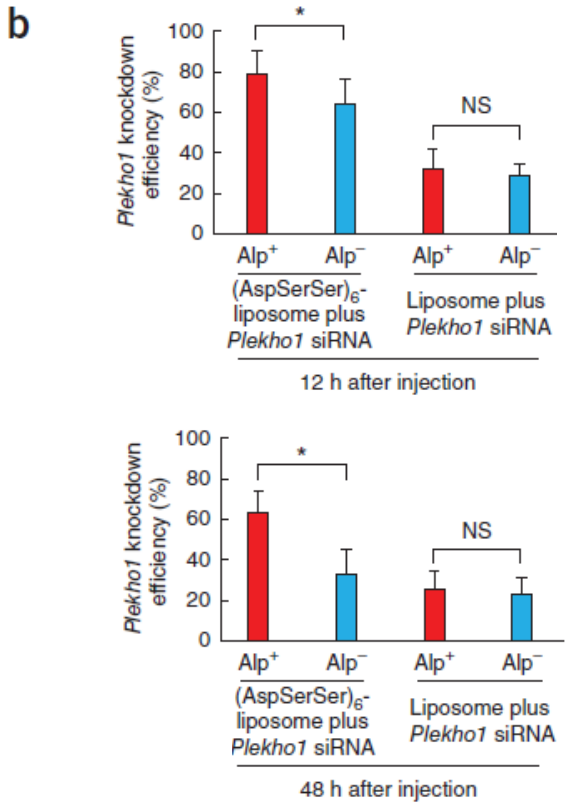


Organ-selective gene knockdown *in vivo*

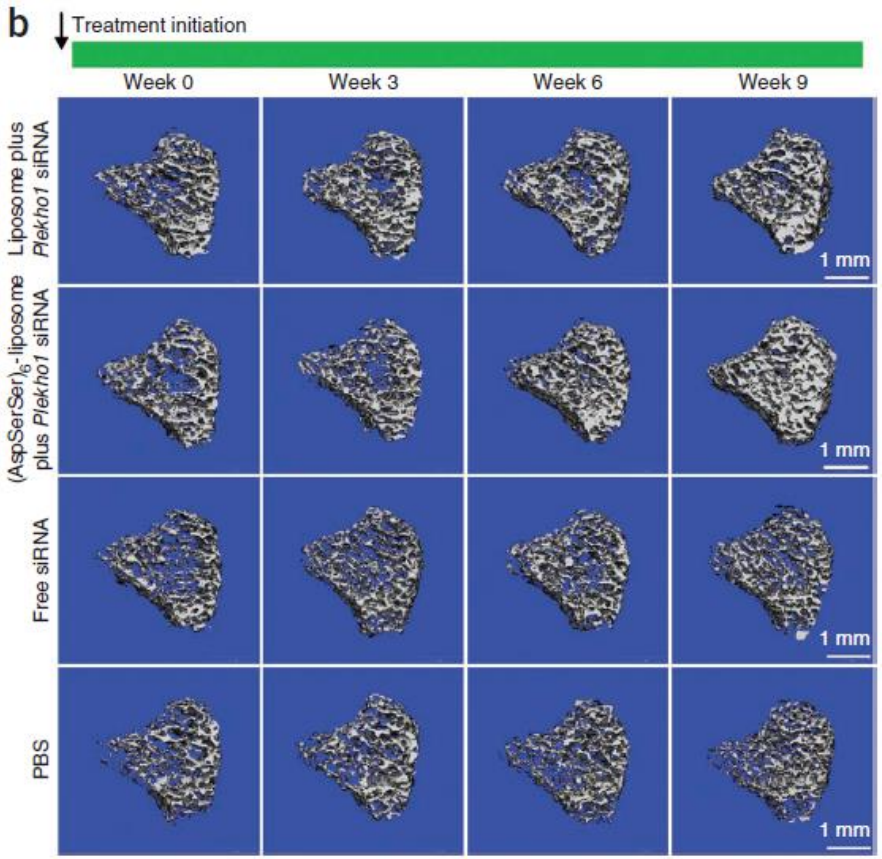


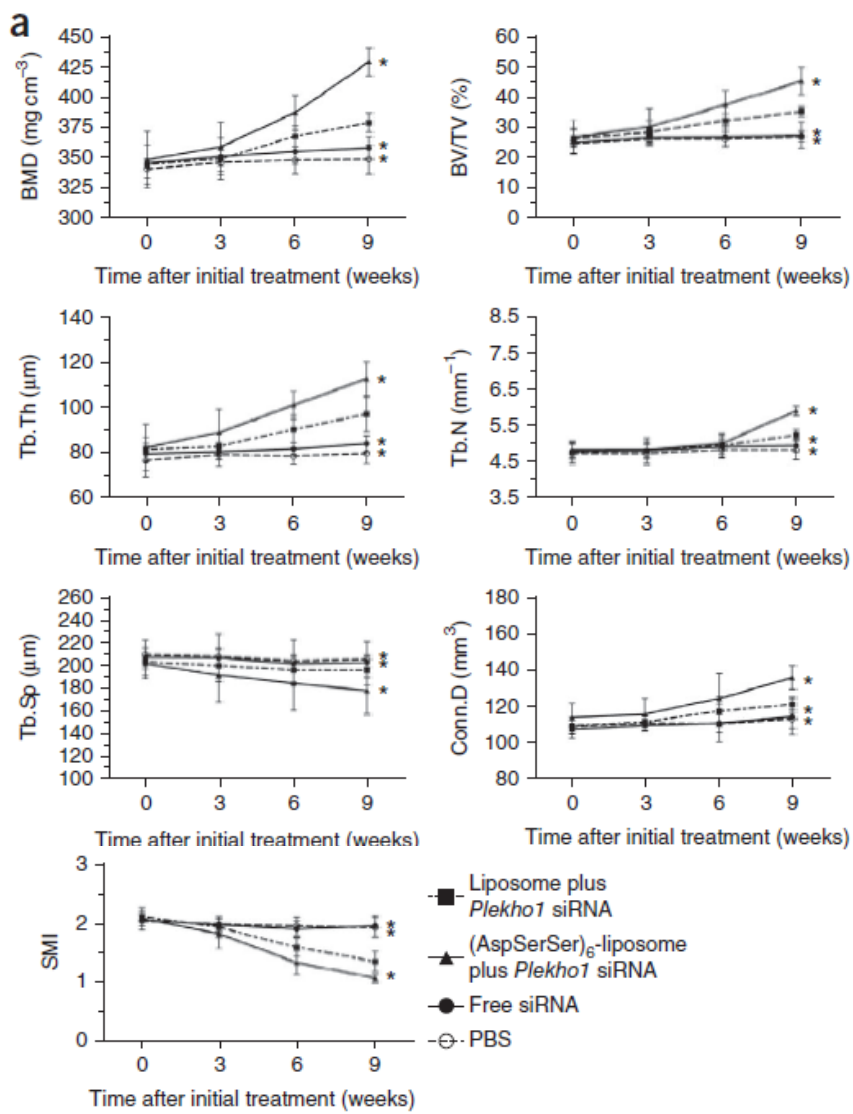
Cell-selective delivery and knockdown efficiency *in vivo*





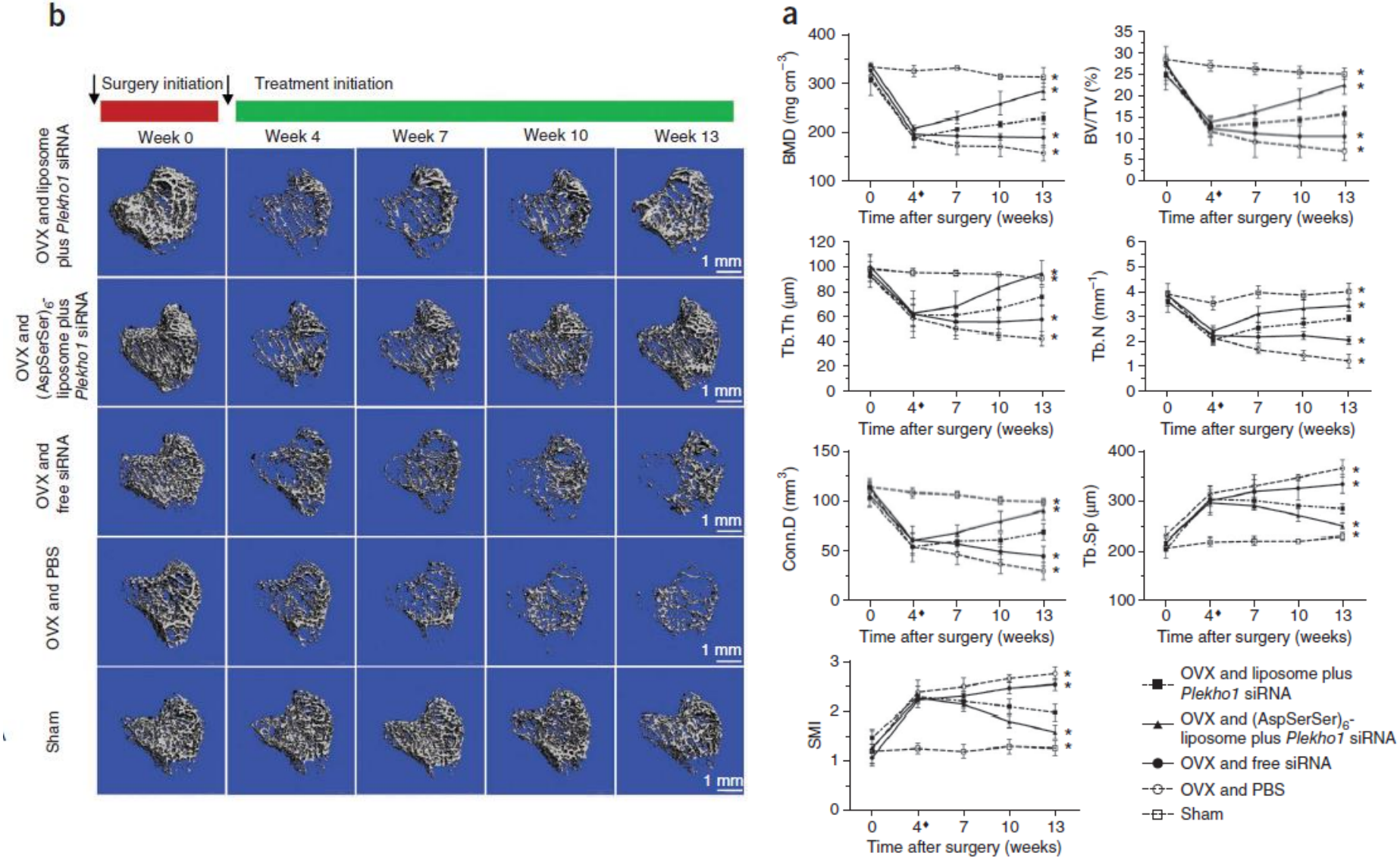
In vivo microCT examinations of the three-dimensional trabecular architecture in healthy rats



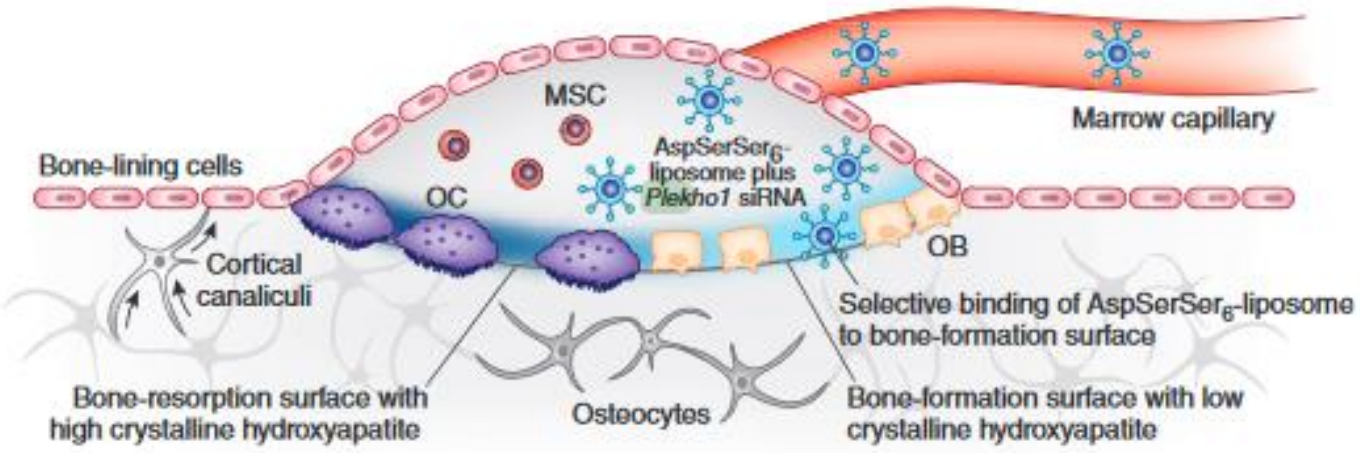


relative bone volume (BV/TV)
trabecular thickness (Tb.Th)
trabecular number (Tb.N)
connectivity density (Conn.D)
structure model index (SMI)
trabecular space (Tb.Sp)

In vivo microCT examination of the three-dimensional trabecular architecture in OVX-treated rats



Summary



Rosen, 2012. *Nature Medicine*

NATURE NANOTECHNOLOGY | ARTICLE

In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight

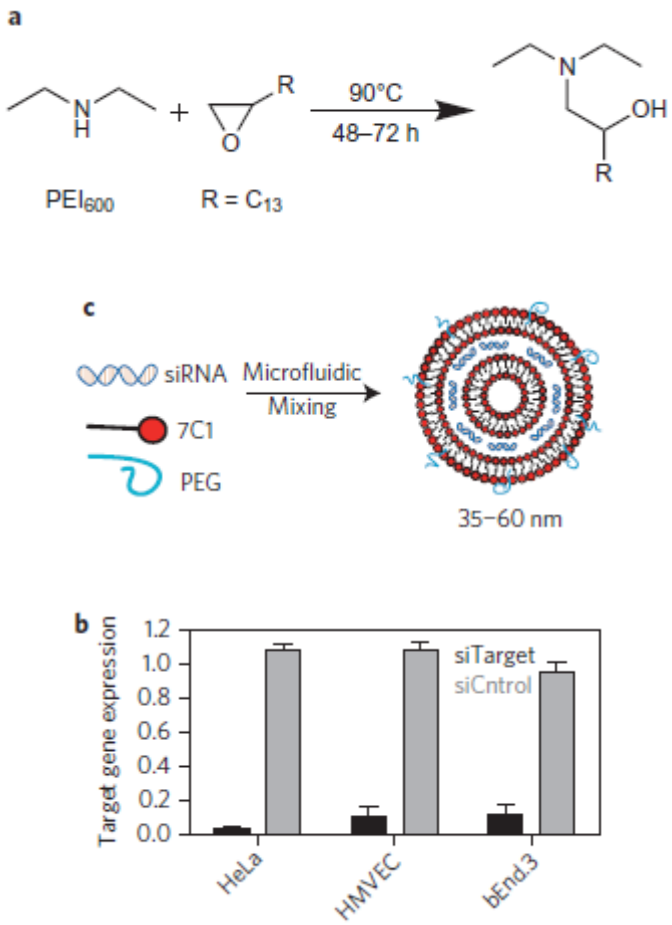
James E. Dahlman, Carmen Barnes, Omar F. Khan, Aude Thiriot, Siddharth Jhunjunwala, Taylor E. Shaw, Yiping Xing, Hendrik B. Sager, Gaurav Sahay, Lauren Speciner, Andrew Bader, Roman L. Bogorad, Hao Yin, Tim Racie, Yizhou Dong, Shan Jiang, Danielle Seedorf, Apeksha Dave, Kamaljeet Singh Sandhu, Matthew J. Webber, Tatiana Novobrantseva, Vera M. Ruda, Abigail K. R. Lytton-Jean, Christopher G. Levins, Brian Kalish + *et al.*

Polyethyleneimine (PEI)

- Polymer class investigated as a gene delivery material
- High molecular weight PEI (approx. 25,000KDa): assoiciated with off-target effects
- Low molecular weight PEI (approx. 600KDa): well tolerated but cannot facilitate siRNA delivery

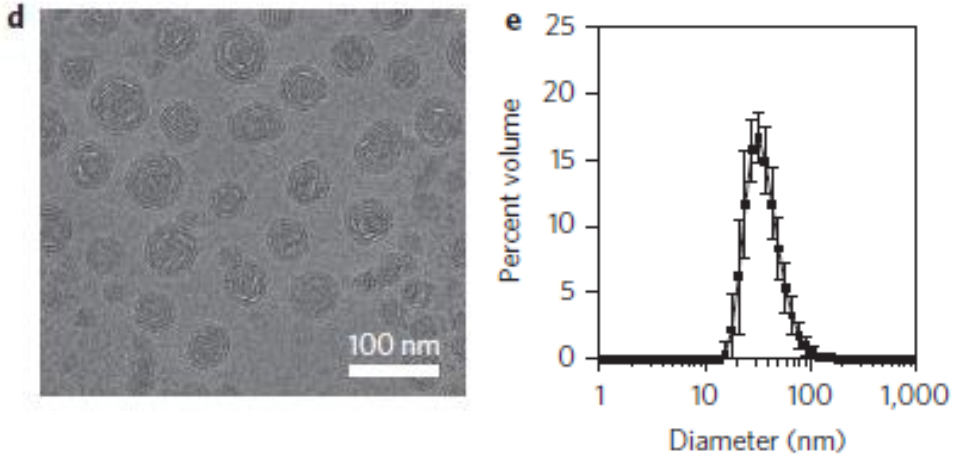
7C1 synthesis

- 7C1 nanoparticles were mixed with
  - $C_{14}PEG_{2000}$
  - siRNA
  - in a high-throughput microfluidic chamber

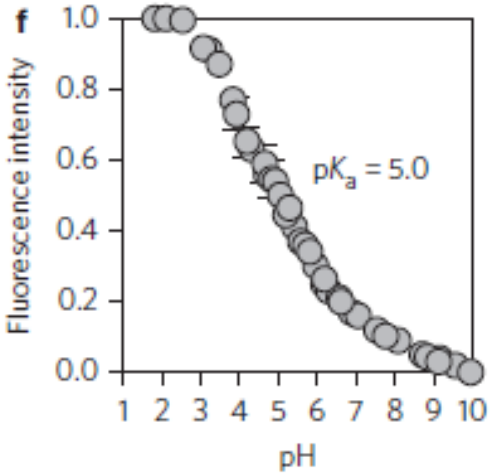


7C1 Characterization

- Average 7C1 hydrodynamic diameter
  - measured by dynamic light scattering, and weighted by volume

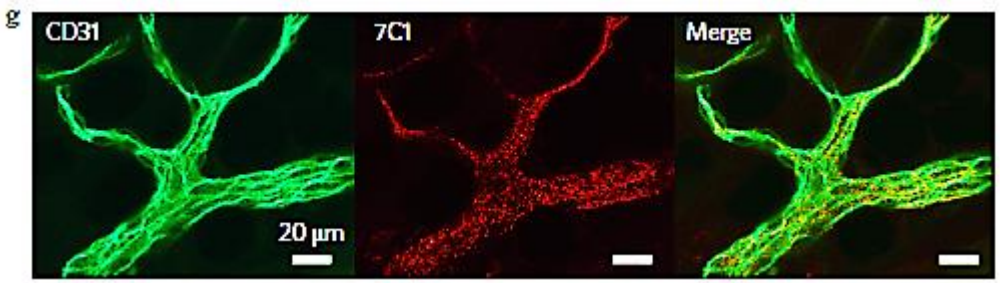


- 6,P-toluidinylnapthalene-2-sulfonate (TNS)
  - fluorescence of formulated 7C1 nanoparticles
  - function of pH (used to measure 7C1 pKa)

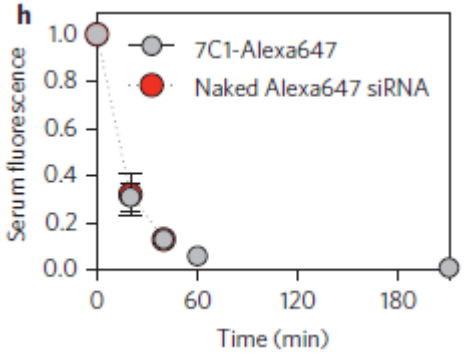


7C1 in vivo biodistribution

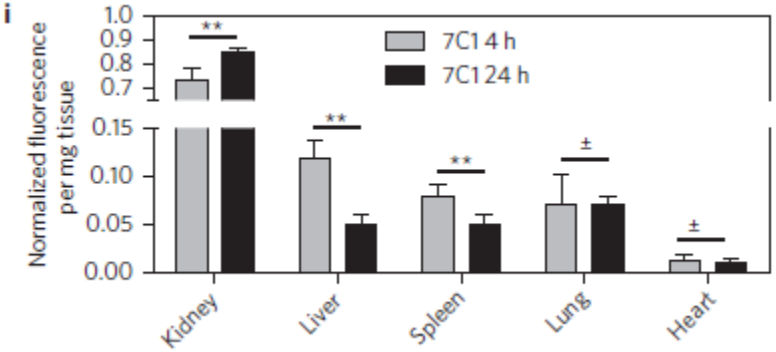
- Alexa647-tagged siRNA complexed to 7C1, 1 h after intravenous injection.



- Serum Cy5.5 concentration following injection with
  - 7C1-Cy5.5 siRNA
  - naked Cy5.5 siRNA.

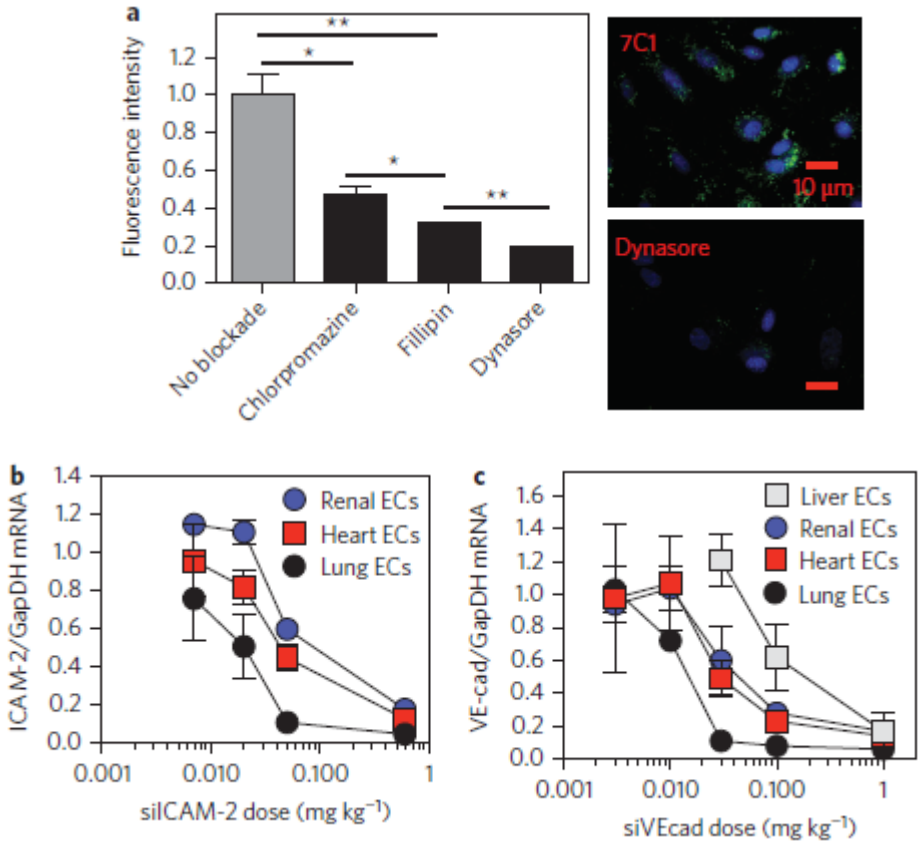


- Time points were selected to measure systemic siRNA accumulation after Cy5.5 was cleared from serum.



7C1 delivers siRNA to endothelial cells

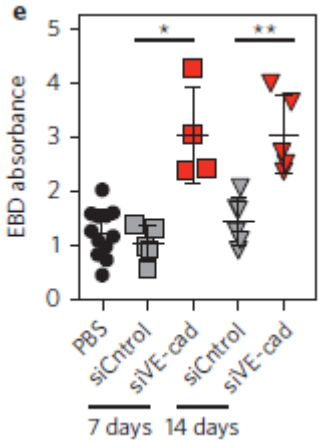
- HMVEC cells following 7C1-Alexa647 treatment in the presence of small molecules inhibiting:
  - clathrin (Chlorpromazine)
  - caveolin (Fillipin)
  - both endocytotic pathways (Dynasore)

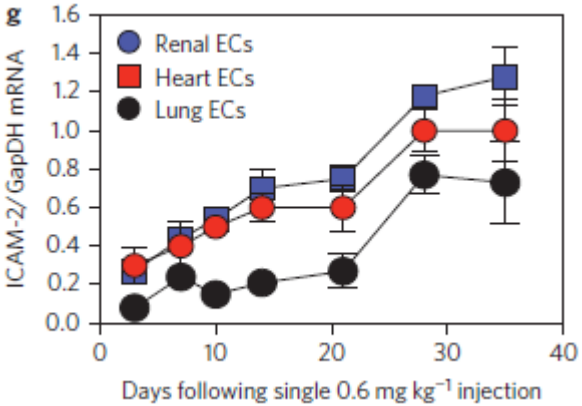


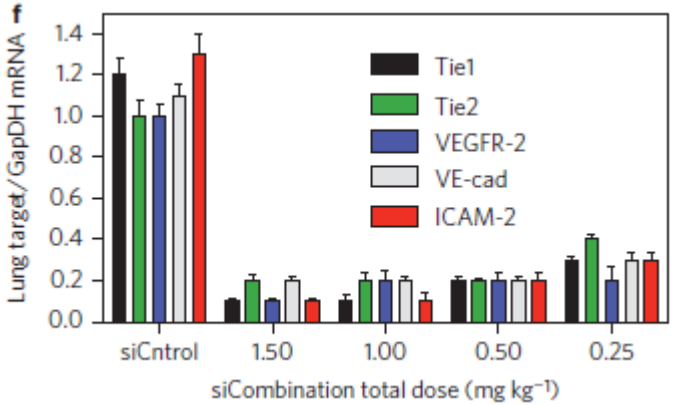
7C1 delivers siRNA to endothelial cells

- Evans Blue Dye (EBD) pulmonary absorbance 7 and 14 days following a 0.6 mg kg^{-1} injection of 7C1-siVEcad.

- Target/GapDH mRNA ratios (normalized to PBS-treated mice) following injection of 7C1 formulated with:
  - siCntrol
  - ICAM-2
  - Tie2
  - VE-cadherin
  - VEGFR2
  - Tie1

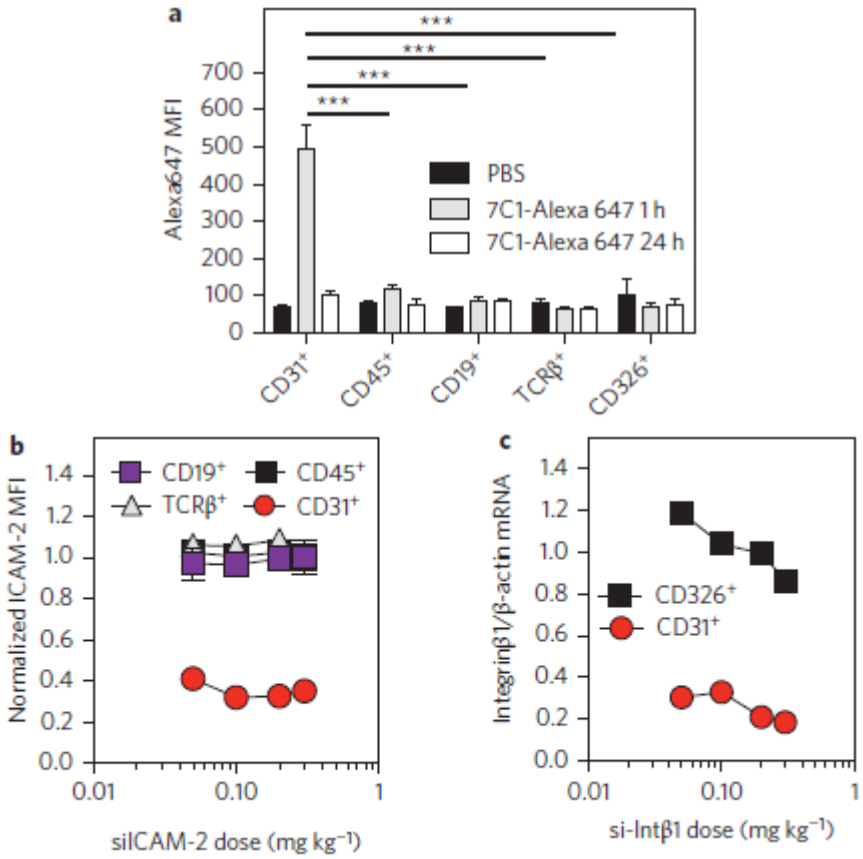






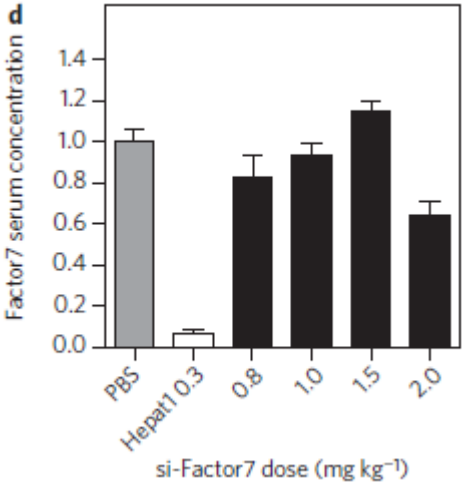
7C1 preferentially delivers siRNA to pulmonary endothelial cells *in vivo*

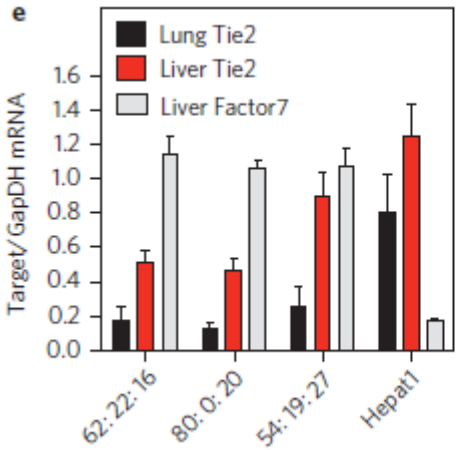
- Isolated from mice after treatment with 7C1 formulated with Alexa647-tagged siRNA.
  - pulmonary endothelial (CD31$^+$)
  - Haematopoietic (CD45$^+$)
  - epithelial (CD326$^+$)
  - B cells (CD19$^+$)
  - T cells (TCRb$^+$)

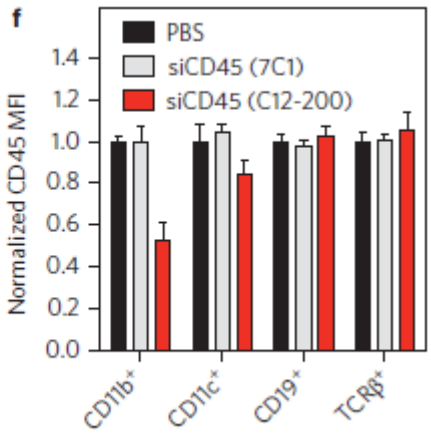


7C1 preferentially delivers siRNA to pulmonary endothelial cells *in vivo*

- Hepatocyte specific: Factor 7
  - two days following treatment with liver-targeting
  - molecule Hepat01-siFactor7 or 7C1-siFactor7.
- Particles were formulated with different 7C1:cholesterol:$C_{14}PEG_{2000}$ molar ratios
  - efficacy can vary with the molar ratio of PEG and cholesterol
- Expression in pulmonary, renal and hepatic endothelium without reducing F7 mRNA expression.
- CD45 median fluorescent intensity following treatment

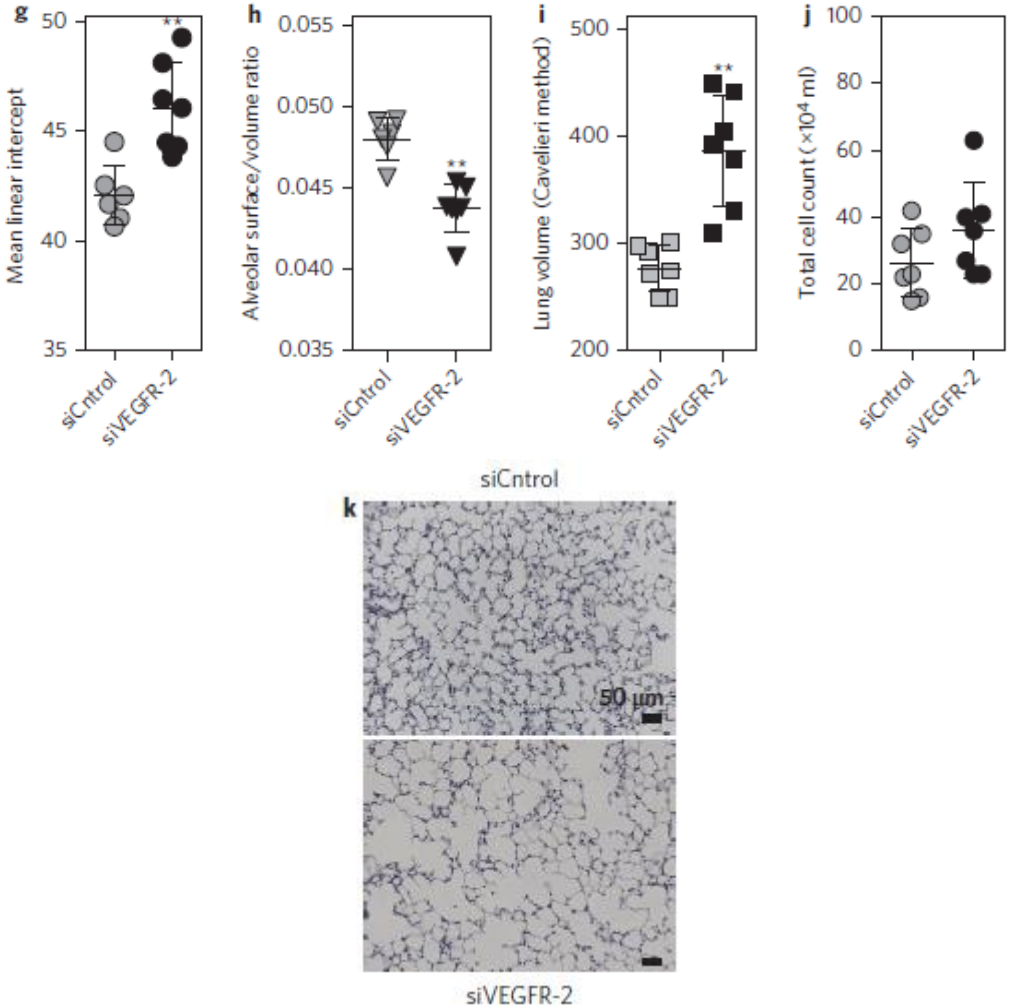






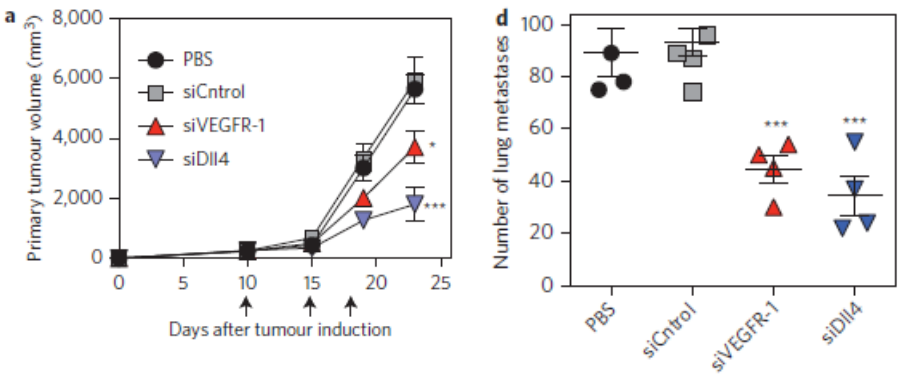
7C1 preferentially delivers siRNA to pulmonary endothelial cells *in vivo*

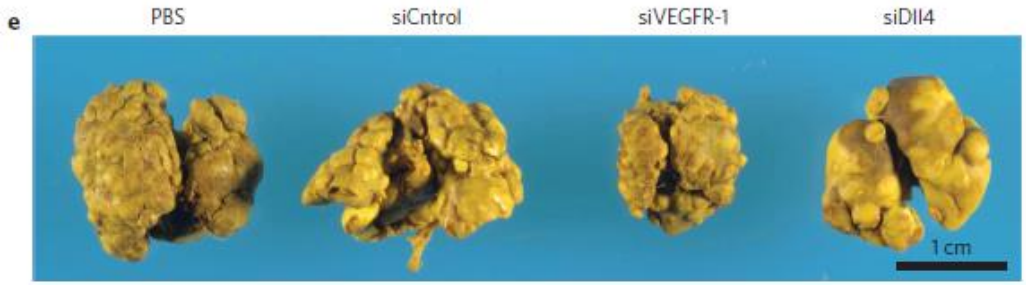
- Mean linear intercept (MLI) between alveoli, pulmonary surface/volume ratio, total volume and pulmonary histology
- following two 0.5 mg kg^{-1} injections of siCntrol or siVEGFR2.
- Increased MLI, alveolar volume, decreased surface/volume ratios and constant infiltrating myeloid cells
- consistent with an induced emphysema-like phenotype



7C1-mediated mRNA silencing modifies endothelial function in vivo

- Primary Lewis lung carcinoma (LLC)
  - growth following three 1.0 mg kg^{-1}
- Number of pulmonary surface metastases following four 1.0 mg kg^{-1} injections
- To measure effects independent of primary tumour growth, animals were not treated until after primary tumour resection.





7C1 in publications

RESEARCH ARTICLE | NANOMEDICINE

RNAi targeting multiple cell adhesion molecules reduces immune cell recruitment and vascular inflammation after myocardial infarction

Hendrik B. Sager[1,*,†], Partha Dutta[1,†], James E. Dahlman[2,3,4,†], Maarten Hulsmans[1], Gabriel Courties[1], Yuan Sun[1], Timo Heidt[1], Claudio Vinegoni[1], Anna Borodovsky[5], Kevin Fitzgerald[5], Gregory R. Wojtkiewicz[1], Yoshiko Iwamoto[1], Benoit Tricot[1], Omar F. Khan[3], Kevin J. Kauffman[3,6], Yiping Xing[2,3], Taylor E. Shaw[2,3], Peter Libby[7], Robert Langer[2,3,4,6], Ralph Weissleder[1,8], Filip K. Swirski[1], Daniel G. Anderson[2,3,4,6,†] and Matthias Nahrendorf[1,9,*,†]

Circulation Research

INTEGRATIVE PHYSIOLOGY

Proliferation and Recruitment Contribute to Myocardial Macrophage Expansion in Chronic Heart Failure

Hendrik B. Sager, Maarten Hulsmans, Kory J. Lavine, Marina B. Moreira, Timo Heidt, Gabriel Courties, Yuan Sun, Yoshiko Iwamoto, Benoit Tricot, Omar F. Khan, James E. Dahlman, Anna Borodovsky, Kevin Fitzgerald, Daniel G. Anderson, Ralph Weissleder, Peter Libby, Filip K. Swirski and Matthias Nahrendorf

Thanks!

https://kam4emet.wordpress.com/tag/food/