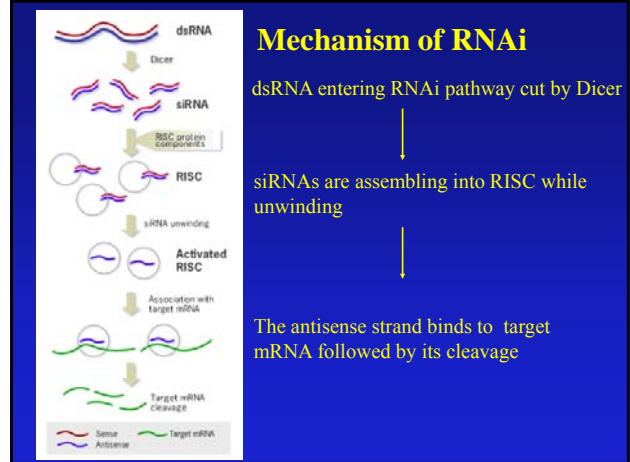


# In vivo imaging of siRNA delivery and silencing

Anna Moore, Ph.D.

Molecular Imaging Lab, MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston



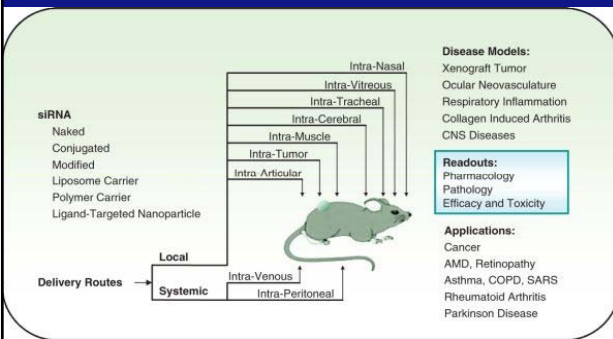
## RNA interference

- RNAi has recently emerged as a powerful potential therapeutic tool
- The main obstacle is delivery of functional siRNA to the target tissue:
  - digestion by nucleases
  - short blood half-life
  - cross cell membrane to achieve adequate target tissue bioavailability
  - assess siRNA delivery non-invasively

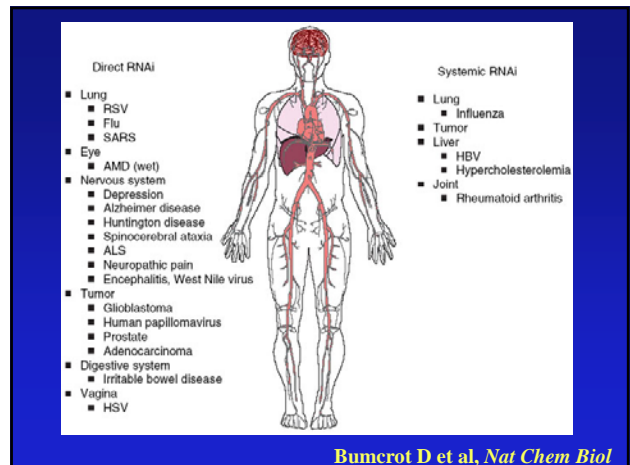
## siRNA delivery

- Hydrodynamic injection (Song et al, Nat Med, 2003; Zender et al, PNAS, 2003)
- Lipid-based agents - polyethylenimine and cationic lipids (Hassani et al, J Gene Med, 2005; Grezeinski et al, 2006; Urban-Klein, Gene Ther, 2005; Schiffelers et al, Nuclear Acids Res, 2004), liposomes (Landen et al, Cancer Res, 2005), lipoplexes (Santel et al, Gene Ther, 2006<sup>1</sup>; Santel et al, Gene Ther, 2006<sup>2</sup>)
- Carriers - atelocollagen (Takei et al, Cancer Res, 2004; Takeshita et al, PNAS, 2005), protamine-Ab fused siRNA (Song et al, Nat Biotech, 2005), cholesterol (Soutschek et al, Nature, 2004), SNALP (Morrissey et al, Nat Biotech, 2005; Zimmermann, Nature, 2006)
- Local delivery (too many to list)

## RNAi as Small Molecule Drugs



Lu and Woodle, *Methods Mol Biol*, 2008



Bumcrot D et al, *Nat Chem Biol*

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### Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles

Mark E. Davis<sup>1</sup>, Jonathan E. Zuckerman<sup>1</sup>, Chung Hang J. Choi<sup>1</sup>, David Seligson<sup>2,3</sup>, Anthony Tolcher<sup>3</sup>, Christopher A. Alabi<sup>1</sup>, Yun Yen<sup>1</sup>, Jeremy D. Heidel<sup>1</sup> & Antoni Ribas<sup>1,4</sup>

siRNA to M2 subunit of ribonucleotide reductase

**a** RRM2 mRNA expression (%)

Group	RRM2 mRNA expression (%)
Control	100
siRNA	~45
siRNA + NP	~25

**b** RRM2 protein expression (%)

Group	RRM2 protein expression (%)
Control	100
siRNA	~25
siRNA + NP	~15

**Table 1 | Overview of high-resolution, small-animal imaging systems**

Technique	Resolution	Depth	Time	Imaging agents	Target*	Cost†	Primary small-animal use	Clinical use
MR	10–100 μm	No limit	Minutes–hours	Gadolinium, dysprosium, iron oxide particles	A, P, M	\$\$\$	Vascular imaging mostly with high soft-tissue contrast	Yes
CT	50 μm	No limit	Minutes	Iodine	A, P	\$\$	Lung and bone imaging	Yes
Ultrasound	50 μm	Millimetres	Minutes	Microbubbles	A, P	\$\$	Vascular and interventional imaging	Yes
PET	1–2 mm	No limit	Minutes	<sup>18</sup> F, <sup>11</sup> C, <sup>15</sup> O	P, M	\$\$\$	Vascular imaging mostly with many different tracers	Yes
SPECT	1–2 mm	No limit	Minutes	<sup>99m</sup> Tc, <sup>111</sup> In chelates	P, M	\$\$	Commonly used to image labeled antibodies, peptides and so on	Yes
FR	2–3 mm	<1 cm	Seconds–minutes	Photoproteins (GFP), NIR fluorochromes	P, M	\$	Rapid screening of molecular events in surface-based tumours	Development
FMT	1 mm	<10 cm	Seconds–minutes	NIR fluorochromes	P, M	\$\$	Quantitative imaging of targeted or ‘smart’ fluorochrome reporters in deep tumours	Development
BLI	Several millimetres	Centimetres	Minutes	Luciferase	M	\$\$	Gene expression, cell and bacterial tracking	No
Intravital microscopy (confocal, multiphoton)	1 μm	<400 μm	Seconds–minutes	Photoproteins (GFP), Fluorochromes	P, M	\$\$\$	All of the above at higher resolution but at limited depths and coverage	Limited development (skin)

\*Primary area that a given imaging modality interrogates: A, anatomical; M, molecular; P, physiological. †Cost of system: \$ <100,000; \$\$ 100–300,000; \$\$\$ >300,000. BLI, bioluminescence imaging; CT, X-ray computed tomography; FMT, fluorescence-mediated tomography; FR, fluorescence reflectance imaging; GFP, green fluorescent protein; NIR, near-infrared; MR, magnetic resonance; PET, positron emission tomography; SPECT, single-photon emission computed tomography.

Massoud and Gambhir, 2003

### siRNA imaging

- Bioluminescence (Lewis et al, Layzer et al, McCaffrey et al, Hassani et al, Kobayashi et al, Pichler et al, Takeshita et al, Bartlett et al.)
- Optical (GFP) (Lewis et al, Sorensen et al, Xia, Pichler et al, Rubinson et al.)
- Nuclear imaging (Liu et al, Merkel et al)

### Bioluminescence imaging of siRNA

Pichler A et al, *Clin Cancer Res*, 2005, 11:4487-4494

### Imaging of siRNA using GFP reporter

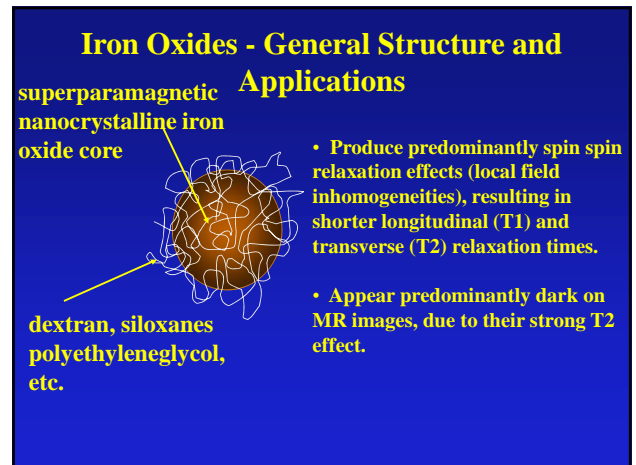
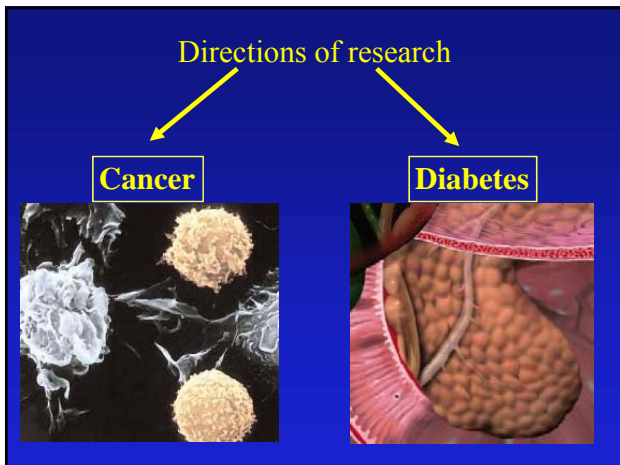
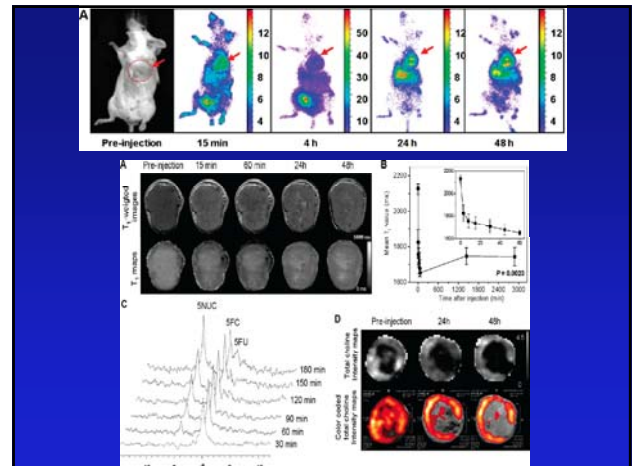
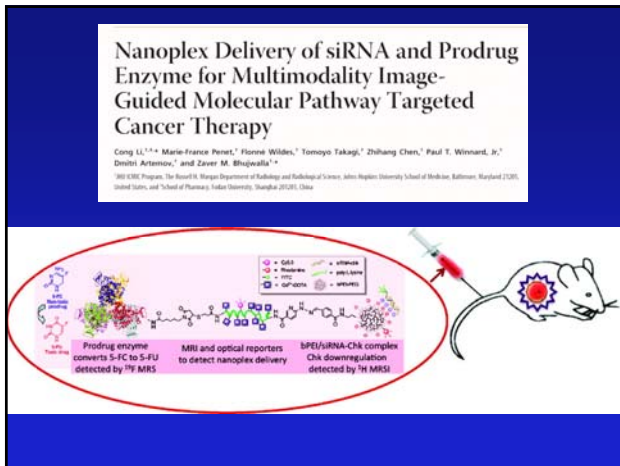
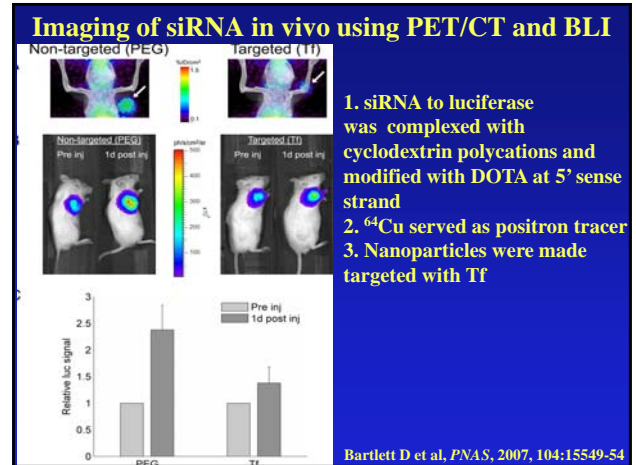
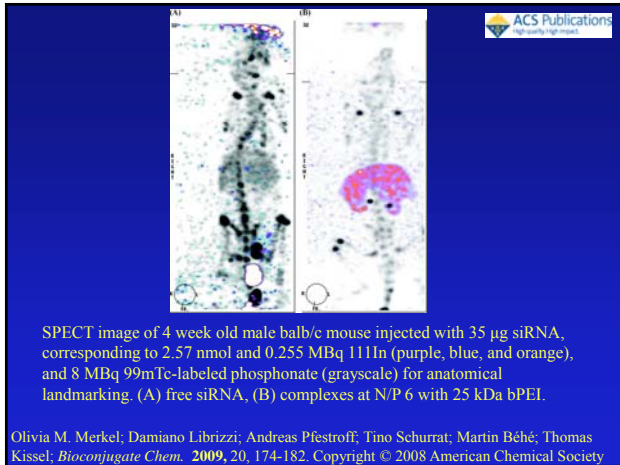
Golzio M et al, *Gene Ther*, 2007, 14:752-59

### Imaging of siRNA in vivo using SPECT

Sense and antisense RNAs against mRNA of the Type I regulatory subunit α of cAMP-dependent protein kinase A

<sup>99m</sup>Tc-labeled RNA injected iv in mice bearing human kidney cancer xenografts

Liu N et al, *Nucl Med Biol*, 2007, 34:399-404



### Magnetic nanoparticles (MN)- properties

Iron core 3-5 nm  
Dextran coating  
diameter = 25 nm  
electron microscopy

Shen T et al, *Magn Reson Med*, 1993, 29:599

### Uptake by various cell types

Type	Species	Iron Uptake $\pm$ SD (ng/10 <sup>6</sup> cells)	Particles per Cell
<b>Primary isolates</b>			
Peritoneal macrophages	Mouse	970 $\pm$ 77	4,850,000
Lymph node lymphocytes	Mouse	19.1 $\pm$ 0.3	95,500
Lymph node lymphocytes	Rat	11.5 $\pm$ 4.3	57,500
Splenic lymphocytes	Mouse	18.7 $\pm$ 0.1	93,500
Splenic lymphocytes	Rat	19.8 $\pm$ 1.3	99,000
Blood lymphocytes	Human	27.9 $\pm$ 3.6	139,500
<b>Tumor cells</b>			
SVEC4-10 endothelial cells	Mouse	118 $\pm$ 25	590,000
C6 glioma	Rat	25.0 $\pm$ 3.0	125,000
9L gliosarcoma	Rat	17.0 $\pm$ 2.0	85,000
J774 sarcoma (macrophage-like)	Rat	310 $\pm$ 28	1,550,000
LX1 small cell lung cancer	Human	29.2 $\pm$ 2.2	145,000
BT 20 breast adenocarcinoma	Human	38.2 $\pm$ 4.0	190,000
MCF-7 breast adenocarcinoma	Human	11.9 $\pm$ 1.6	60,000
HBL100 breast adenocarcinoma	Human	33.2 $\pm$ 0.1	165,000
D4.475 breast adenocarcinoma	Human	21.1 $\pm$ 0.8	105,000
LS1747 colon adenocarcinoma	Human	60.0 $\pm$ 1.5	300,000
U87 glioma	Human	18.4 $\pm$ 0.5	90,000

Note.—Uptake was measured after 1 hour incubation of 100,000 cells in the presence of 100  $\mu$ g of 125I-labeled LCDIO.

Moore A, et al. *Radiology* 2000, 214:568-574

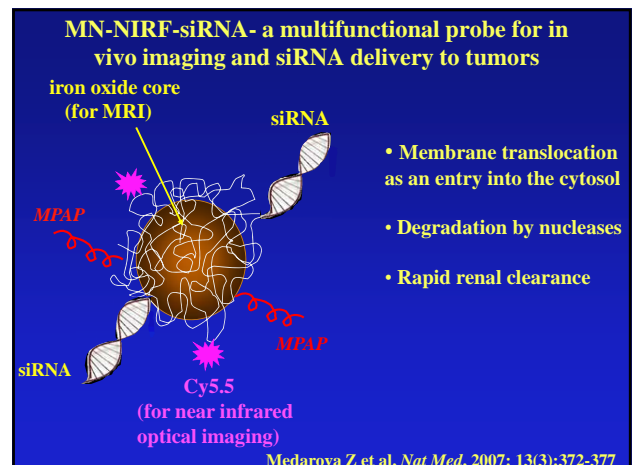
### Localization to the endosomes/lysosomes

p. macrophages  
C6 glioma cells

Moore A et al. *J Mag Reson Imag*, 1997, 7:1140-1145

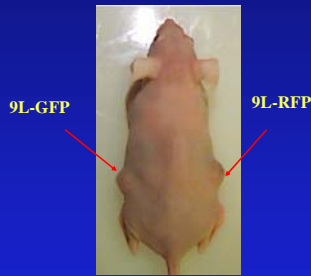
### In vivo imaging of siRNA delivery and silencing in tumors

- ### Goals
1. Deliver siRNA to tumors using nanoparticles as carriers
  2. Image the delivery of siRNA to tumors by MRI and near-infrared optical imaging (NIRF)
  3. Image the silencing effect resulting from siRNA delivery to tumors

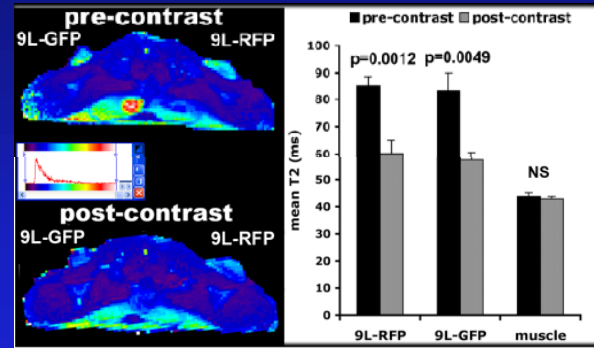


### Experimental setup

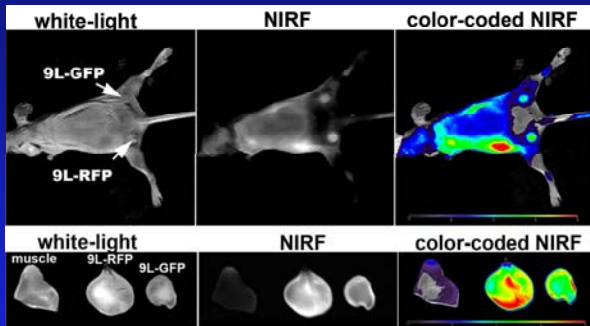
- Bi-lateral tumors:
- 9L-GFP and 9L-RFP
- Probe MN-NIRF-siGFP (10mg Fe/kg; I.V.)
- *In vivo* imaging: MRI and NIRF imaging 24 h post injection



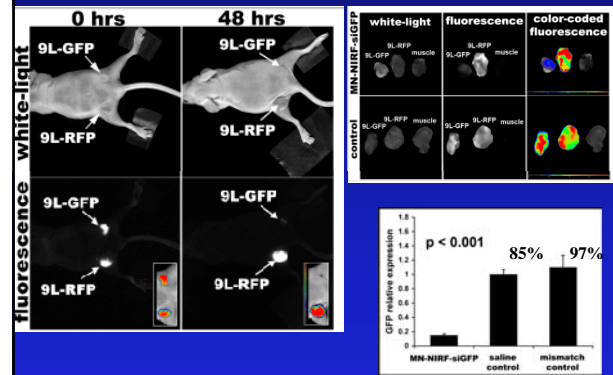
### In vivo MR imaging of siRNA delivery



### In vivo NIRF imaging of siRNA delivery



### In vivo imaging of gene silencing

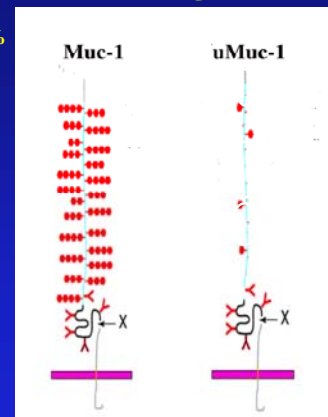


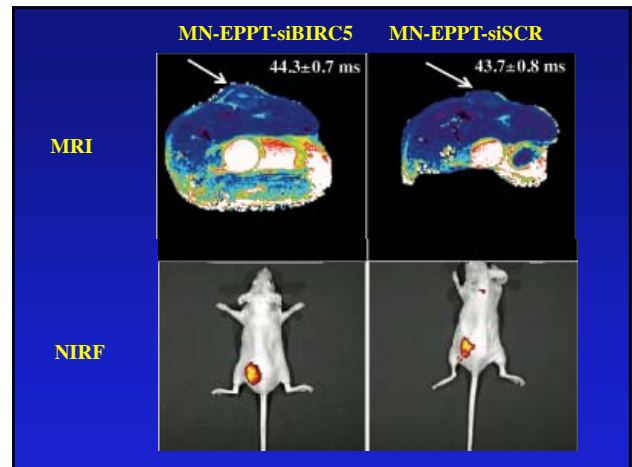
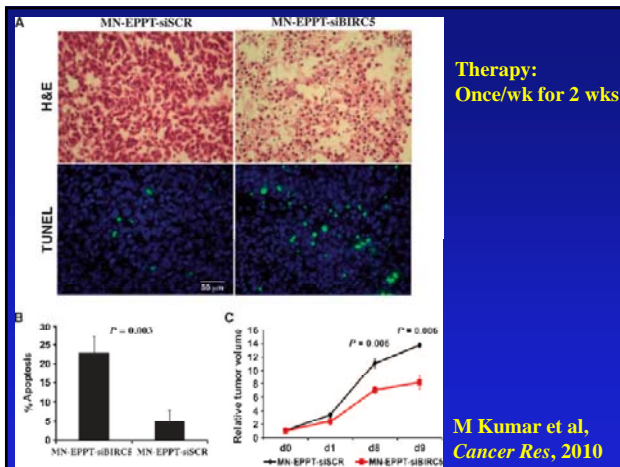
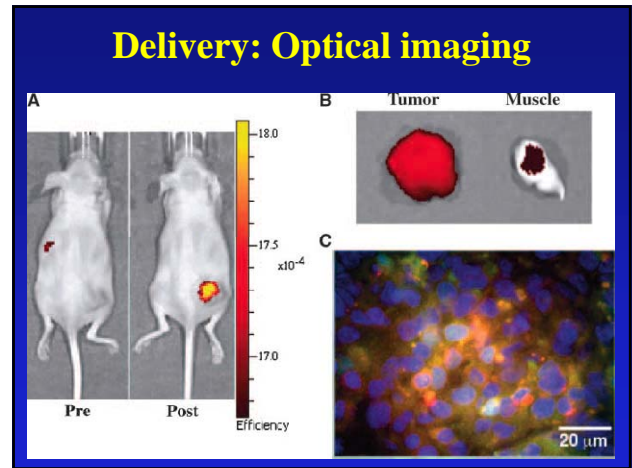
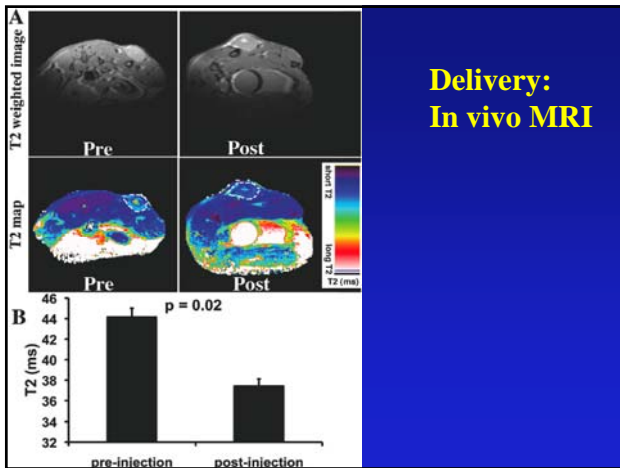
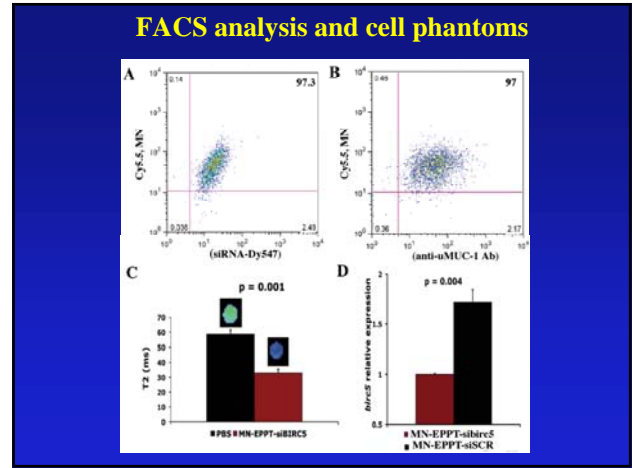
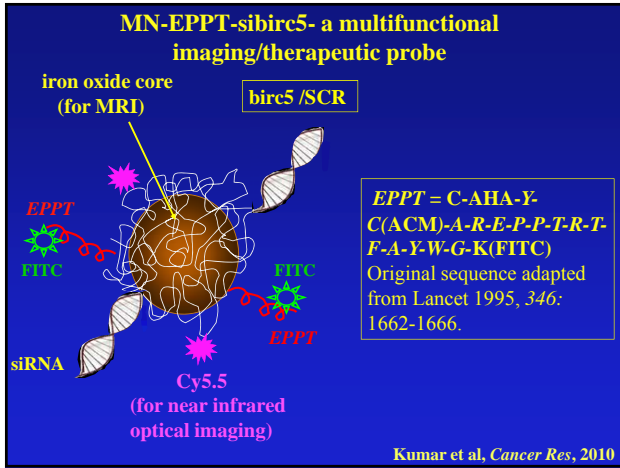
### Next step:

- Therapeutic application
- Increase siRNA bioavailability by specific targeting to tumors

### uMUC-1 tumor antigen

- present on over 50% of human cancers (breast, pancreatic, colorectal, etc.)
- overexpressed on tumor cells
- underglycosylated on tumor cells

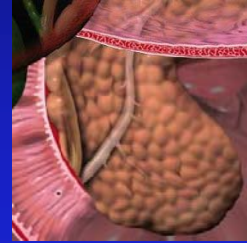




### Summary

- MN-NIRF-siRNA is a promising new tool for the delivery of siRNA to tumors and concurrent imaging of the delivery process
- Therapeutic and imaging implications of this study are significant. Knocking down therapeutic targets, e.g. survivin, can serve to mediate tumor regression, reduced potential for metastasis, enhanced chemosensitivity.
- Application to other pathologies

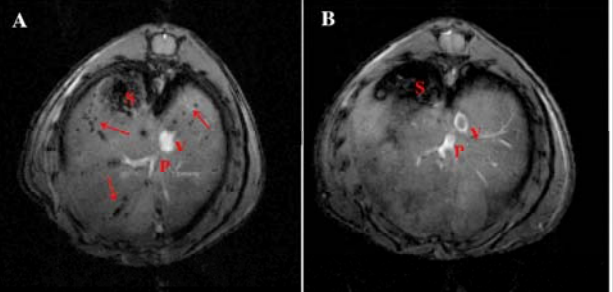
### Imaging of siRNA delivery and silencing in pancreatic islets



### Islet transplantation

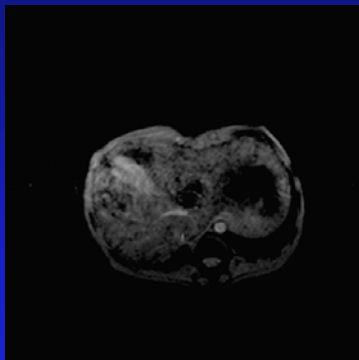
- Type 1 diabetes is caused by autoimmune destruction of insulin-producing beta-cells
- Islet transplantation as a clinical method to achieve insulin independence
- Severe graft loss occurs 2 weeks after transplantation
- Viability and functionality of the transplant is unknown
- Can transplanted islets be visualized?

### In vivo MRI of intrahepatic transplantation of islets labeled with magnetic nanoparticles



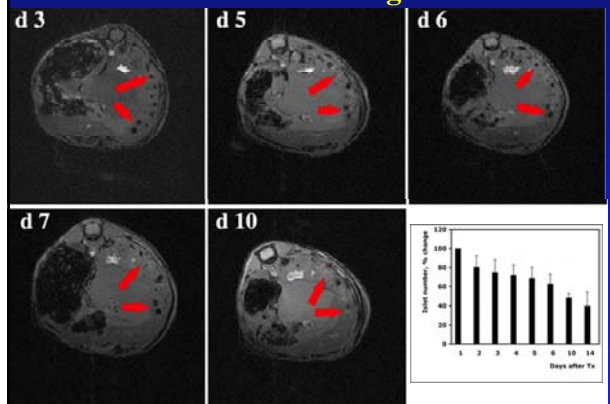
Evgenov N et al, *Nat Med*, 2006  
Evgenov N et al, *Diabetes*, 2006

### In vivo MRI of labeled allogeneic islets transplanted in the liver



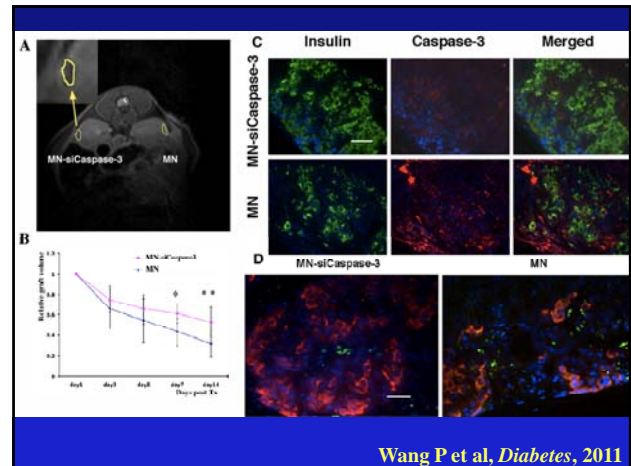
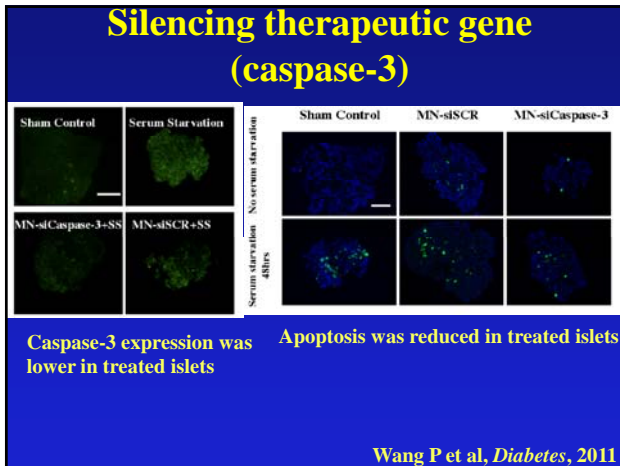
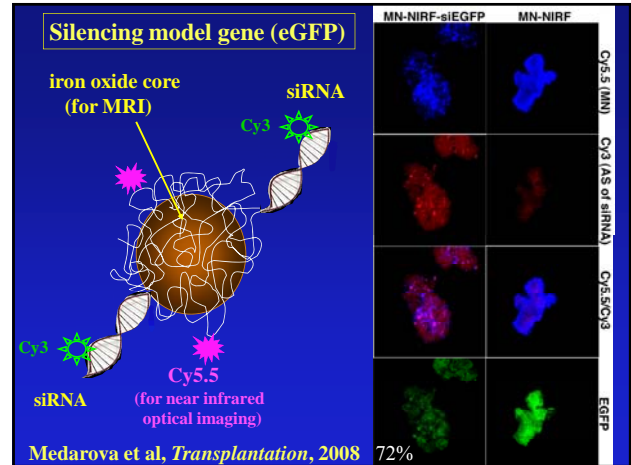
Medarova et al, *Transplantation*, 2009

### In vivo MRI monitoring of islet loss



Evgenov N et al, *Diabetes*, 2006

Goal of the study:  
**To combine siRNA therapy with MR imaging for improved survival of islet grafts with concurrent in vivo monitoring**



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