

## Imaging of siRNA delivery and silencing in tumors

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## RNA interference

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

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## siRNA delivery

- Hydrodynamic injection (Song et al, Nat Med, 2003; Zender et al, PNAS, 2003)
- Lipid-based agents - polyethyleimine 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), lysosomes (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)

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**Direct RNAi**

- Lung
  - RSV
  - Flu
  - SARS
- Eye
  - AMD (wet)
- Nervous system
  - Depression
  - Alzheimer disease
  - Huntington disease
  - Spinocerebral ataxia
  - ALS
  - Neuropathic pain
  - Encephalitis, West Nile virus
- Tumor
  - Glioblastoma
  - Human papillomavirus
  - Prostate
  - Adenocarcinoma
- Digestive system
  - Irritable bowel disease
- Vagina
  - HSV

**Systemic RNAi**

- Lung
  - Influenza
  - Tumor
- Liver
  - HBV
  - Hypercholesterolemia
- Joint
  - Rheumatoid arthritis

**Bumcrot D et al, Nat Chem Biol, 2006**

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**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, dipyridim, iron oxide particles	A, P, M	\$\$\$	Versatile imaging modality 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	\$\$\$	Versatile imaging modality 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
FI	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	Luciferins	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 resolutions but all limited depths and coverage	Limited development (skin)

\*Primary area that a given imaging modality interrogates; A, anatomical; M, molecular; P, physiological. †Cost of system: \$, <10,000; \$\$, 100–500,000; \$\$\$, >500,000. BLI, bioluminescence imaging; CT, X-ray computed tomography; FMT, fluorescence-mediated molecular tomography; FI, fluorescence reflectance imaging; GFP, green fluorescent protein; NIR, near-infrared; MRI, magnetic resonance; PET, positron emission tomography; SPECT, single-photon emission computed tomography.

**Massoud and Gambhir, 2003**

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## 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, Nucl Med Biol, 2007)**

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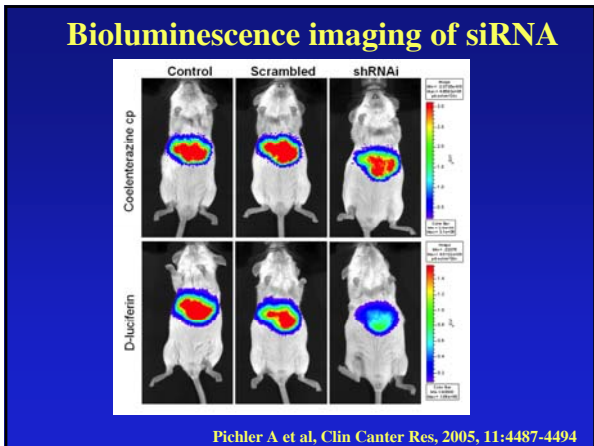
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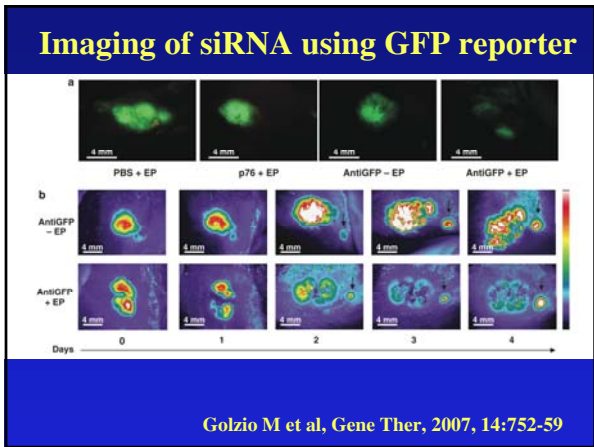
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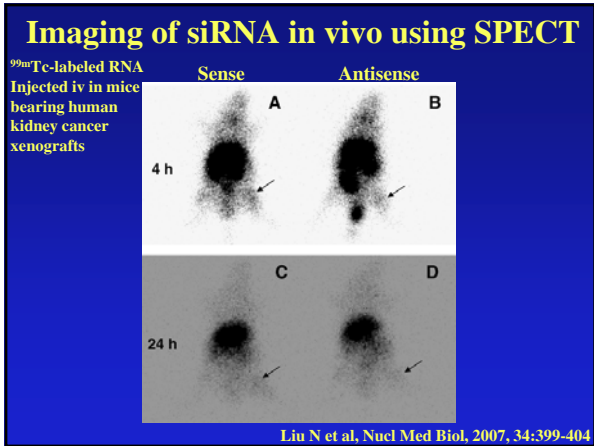
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### Imaging of siRNA in vivo using PET/CT and BLI

1. siRNA to luciferase was complexed with cyclodextrin polycations and modified with DOTA at 5' sense strand  
 2. <sup>64</sup>Cu served as positron tracer  
 3. Nanoparticles were made targeted with Tf

Bartlett D et al, *PNAS*, 2007, 104:15549-54

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### Goals

1. Deliver siRNA to tumors
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

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### MN-NIRF-siRNA- a multifunctional probe for in vivo imaging and siRNA delivery to tumors

- Membrane translocation as an entry into the cytosol.
- Degradation by nucleases
- Rapid renal clearance
- Adequate bioavailability at the target tissue.

Medarova Z et al, *Nature Medicine*, 2007; 13(3):372-377

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### 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



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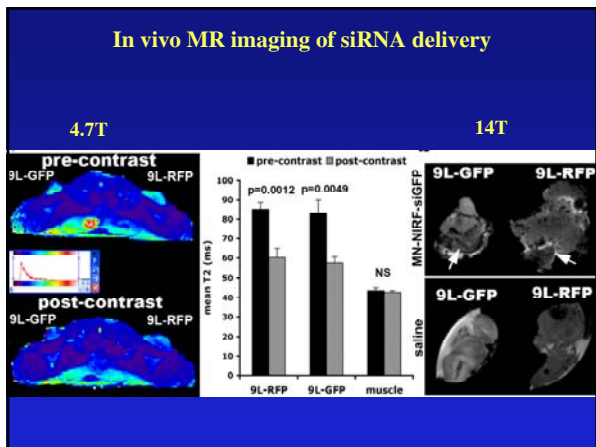
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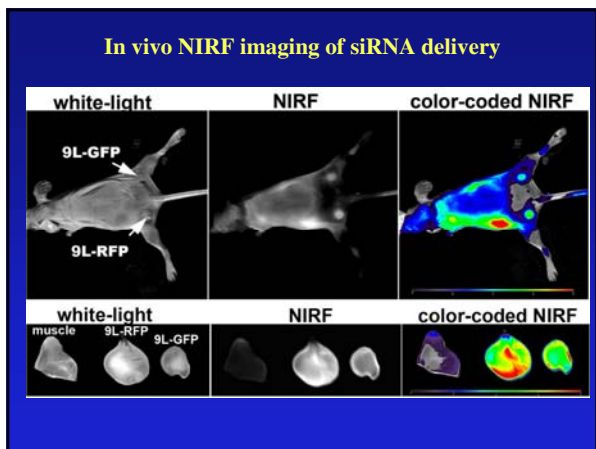
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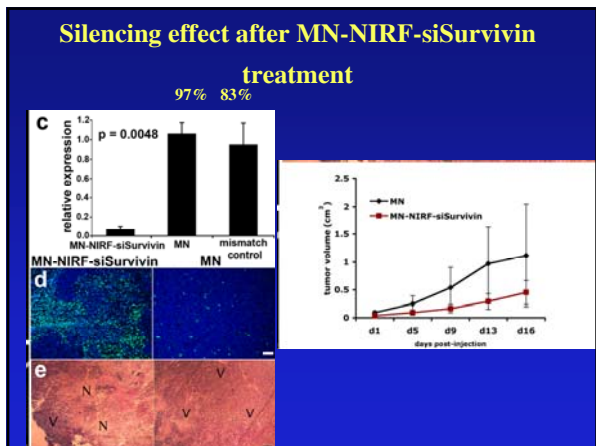
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### Summary

- 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, enhanced chemosensitivity, reduced potential for metastasis, etc.

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### Future directions

- Comprehensive investigation of the mechanism of MN-NIRF-siRNA action
- Improvement of bioavailability
- Combination treatment
- Application to other pathologies

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