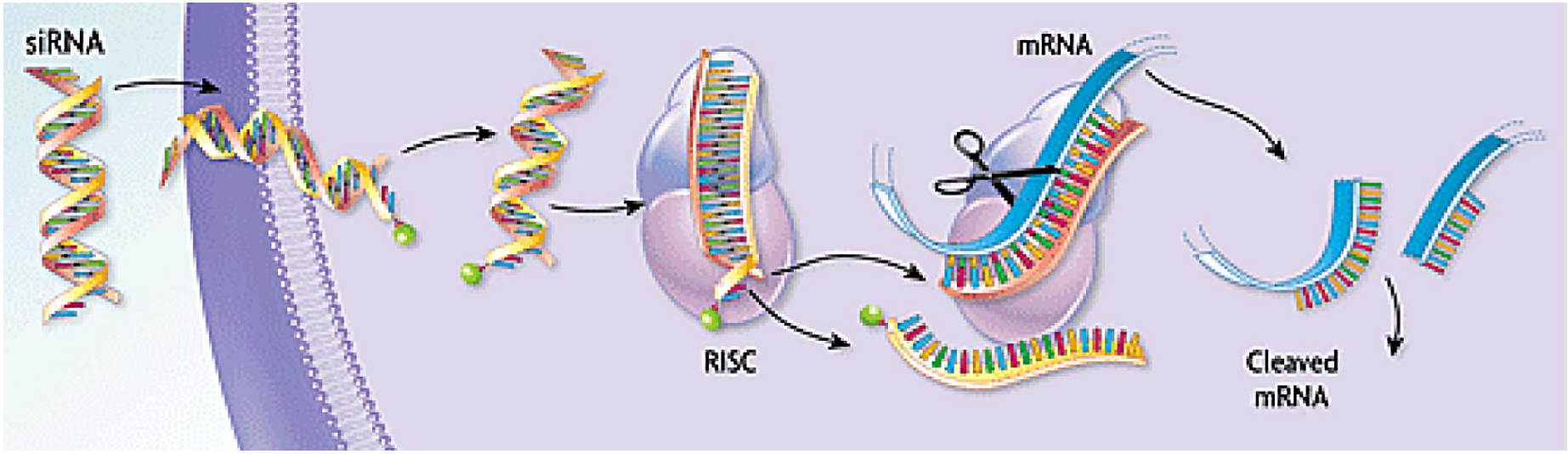
The Silent Treatment: A progress report on RNA therapeutics



Judy Lieberman
Program in Cellular and Molecular Medicine, Boston Children's Hospital, Harvard Med School

Disclosure: I am on the Scientific Advisory Board of Alnylam Pharmaceuticals.

Exogenous small interfering RNAs (siRNA) can hijack the RNA interference machinery

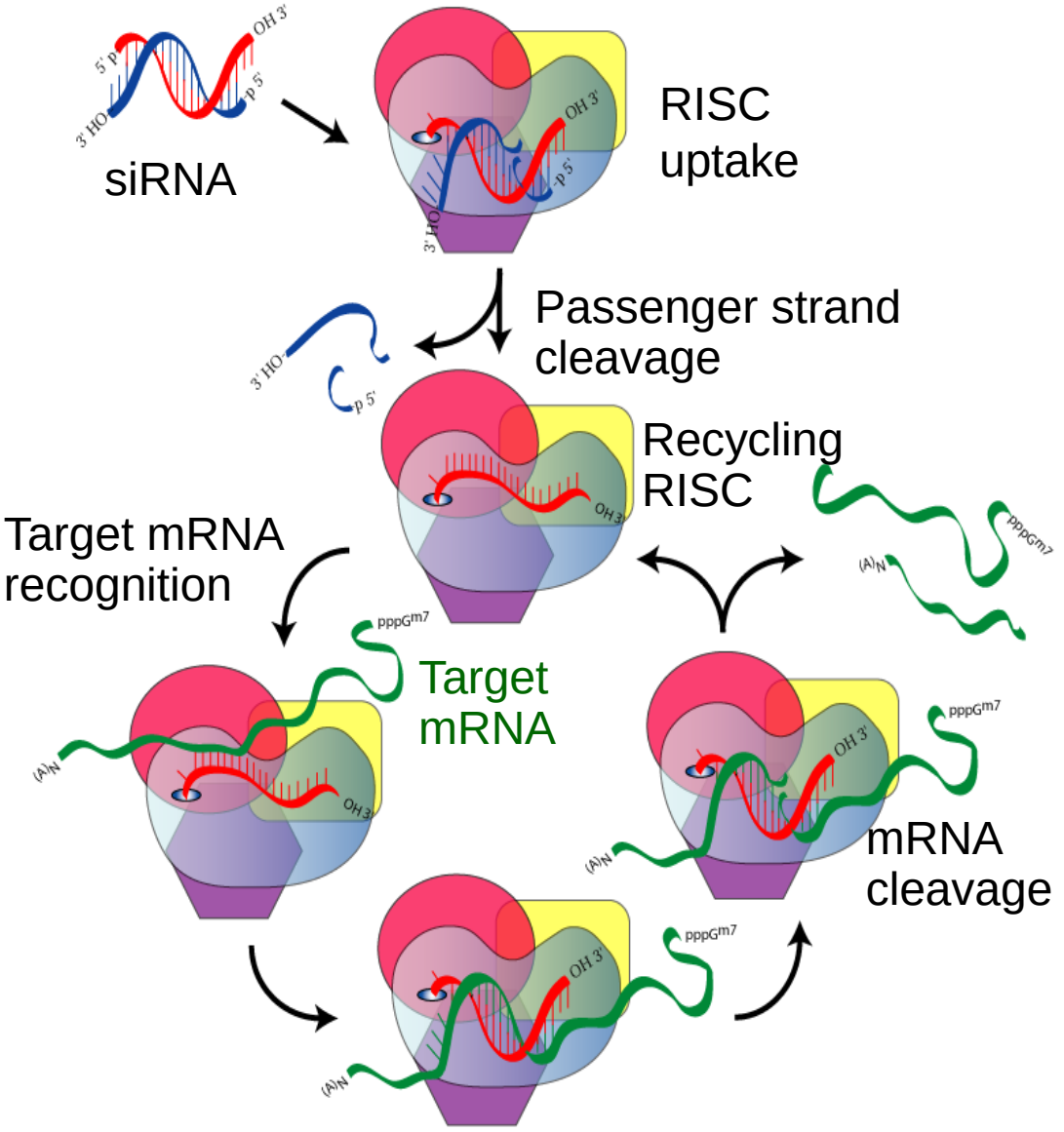
The Catalytic RISC RNA Cycle

The same small RNA is used over and over.

The active siRNA strand is stable in the RISC for weeks-months.

<1000 siRNAs/cell cause complete KD.

Therefore, potent and durable silencing.



Why the excitement about RNAi therapy?

1. Highly specific gene silencing
2. All genes are druggable
3. Straightforward to identify active drugs, cheap to make compared to biologics
4. Very rapid development - 15 mo to Phase I
5. Generally well tolerated
6. Significant and durable gene knockdown and signs of clinical benefit even in small early phase studies

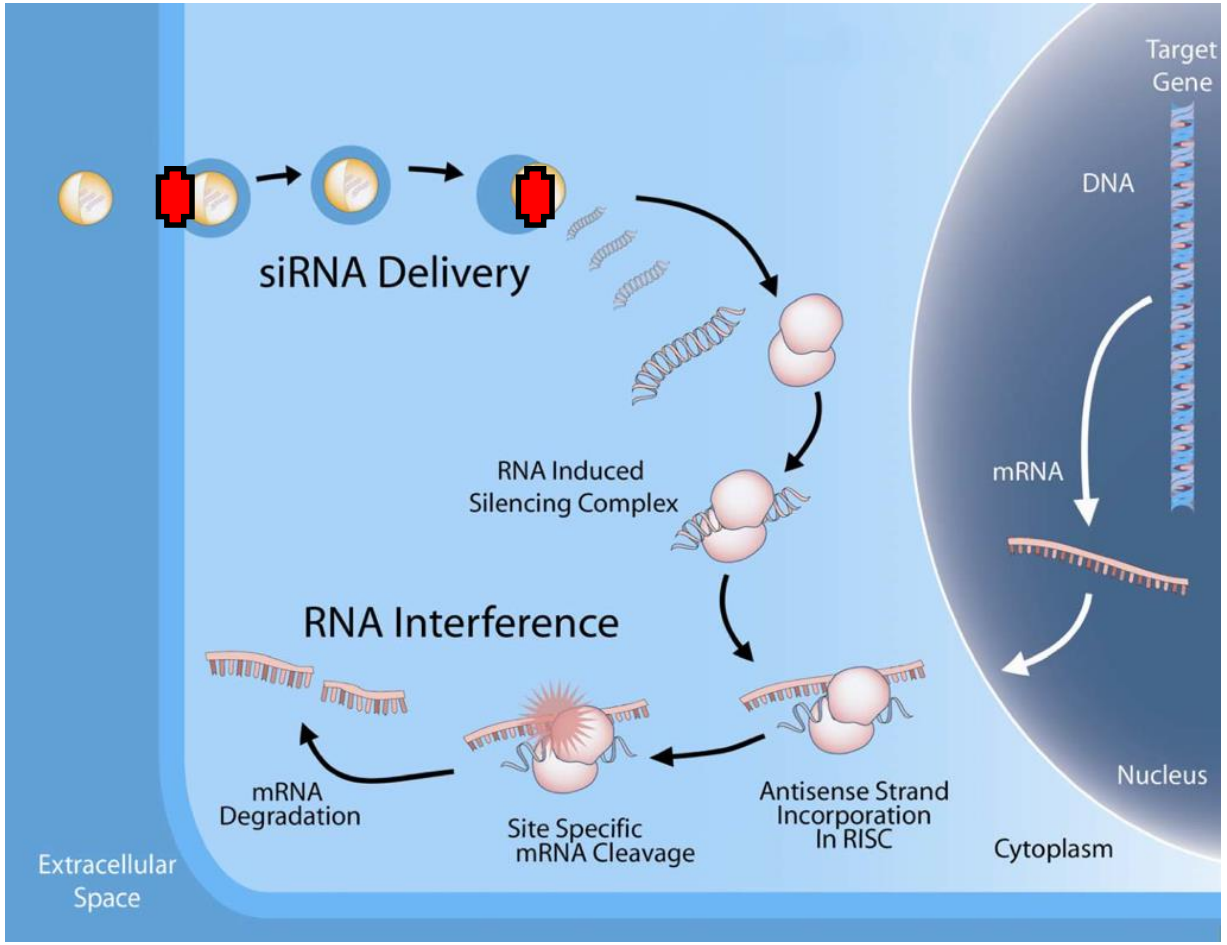
It is only 17 years since RNAi was shown to work in mammals.

A phase III study already showed dramatic benefit and safety.

The first siRNA drug will likely be approved this year.

The biggest challenge to turning siRNAs into drugs is

Delivery



To be active, an siRNA drug needs to get to the cytosol. Naked siRNAs do not get taken up into cells. Even in phagocytic cells, RNAs don't get out of endosomes.

2 bottlenecks -

1. intracellular delivery of RNAs across the target cell membrane into endosomes
2. release from endosomes into the cytosol where the RNAi machinery is

Potential sources of toxicity

On target toxicity – potent knockdown of target gene could have anticipated or unanticipated toxicity

- One way to minimize is to choose a target that does not cause disease in humans or mice bearing homozygous mutations (i.e. *PCSK9*)

Off target effects (miRNA effect)

- Can be reduced by lowering specific siRNA concentration (for example by using cocktails), choosing another sequence to target, chemical modifications, utility of RNA-seq to predict off-target effects?

Innate immune activation

- By binding to TLR3, TLR7 or RIG-I – abolished by chemical modifications
- Other immune toxicities linked to delivery mechanism – flu-like sx, inflammatory cytokines, complement activation – mechanisms not well understood
- Toxicity of delivery mechanism
- All particles (LNPs, nanoparticles, viruses) cause some cytokine elevation independently of nucleic acid cargo – mechanism unknown

Accumulation of nonbiodegradable modified nucleic acids or lipids

- Efforts to design biodegradable constructs

Antibodies to siRNAs

- Antibodies not made to RNA, but could be made to RNA-protein complexes; not a known problem, but not sure anyone has looked carefully

Interference with endogenous miRNA pathway

- Not enough siRNA gets into cell to interfere with RISC function

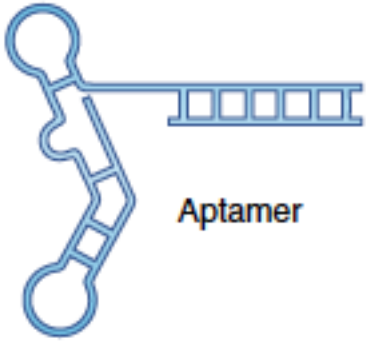
Turning siRNAs into drugs

- Identify gene target for disease
- Select siRNA: in silico prediction, experimental gene walk, check for off-target sequences, find evolutionarily conserved sequence (better for drug development in small animal and nonhuman primate tox and efficacy studies)
- Optimize chemical modifications to enhance stability in serum from exo- and endo-ribonucleases, suppress innate immune activation, improve specific activity
- Select delivery platform

RNA-based drug delivery platforms

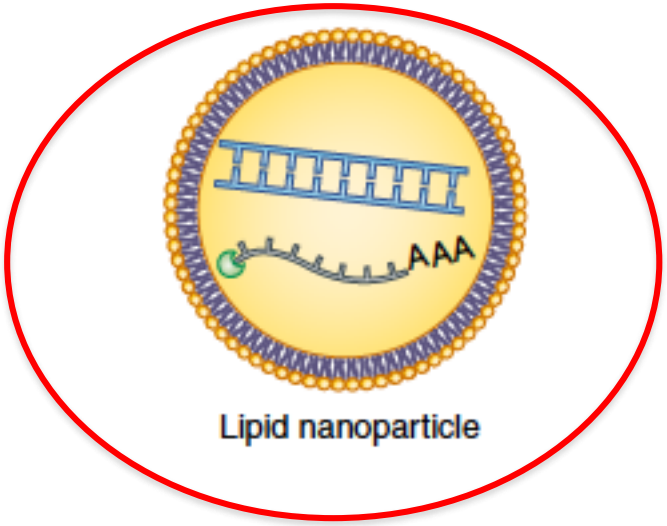
Uptake of naked ssASO

Conjugation to targeting ligand



Aptamer

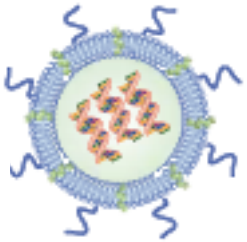
Nucleic acid–peptide complexes



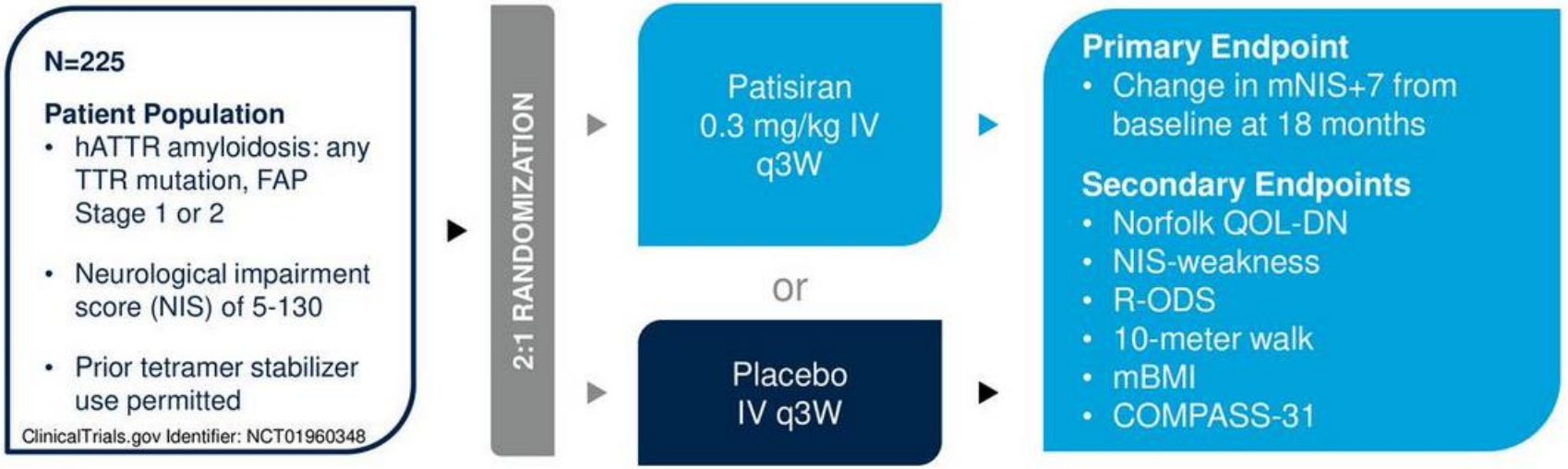
Lipid nanoparticle

Nanoparticle

Apollo Patisiran Phase 3 Study



Knockdown transthyretin gene (*TTR*), which when mutated aggregates in cells, to treat familial amyloidotic polyneuropathy using LNP-encapsulated siRNAs



Primary Endpoint (18 mo.)		p-value
mNIS+7	Neuropathy score	9.26×10^{-24}

Secondary Endpoints (18 mo.)		p-value
Norfolk-QoL	Quality of life	1.10×10^{-10}
NIS-W	Motor strength	1.40×10^{-13}
R-ODS	Disability score	4.07×10^{-16}
10MWT	Walking	1.88×10^{-12}
mBMI	Nutrition	8.83×10^{-11}
COMPASS-31	Autonomic nerve function	0.0008

Safety	Patisiran	Placebo
Adverse Events	96.6%	97.4%
Serious Adverse Events	36.5%	40.3%
Deaths	4.7%	7.8%
Discontinuations from Treatment	7.4%	37.7%
Discontinuations from Treatment due to AEs	4.7%	14.3%
AEs in ≥10% of patients, seen more frequently in patisiran compared with placebo:		
• Peripheral edema*	29.7%	22.1%
• Infusion related reactions*	18.9%	9.1%

RNA-based drug delivery platforms

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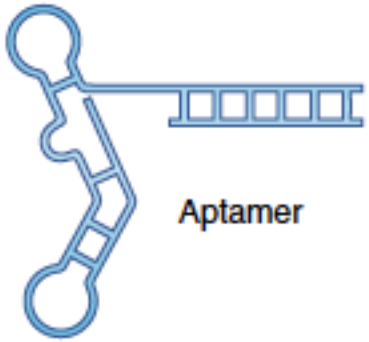
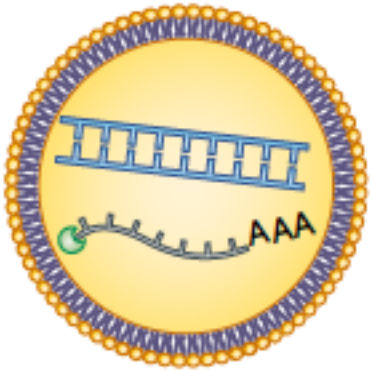
Conjugation to targeting ligand



Aptamer

Nucleic acid–peptide complexes



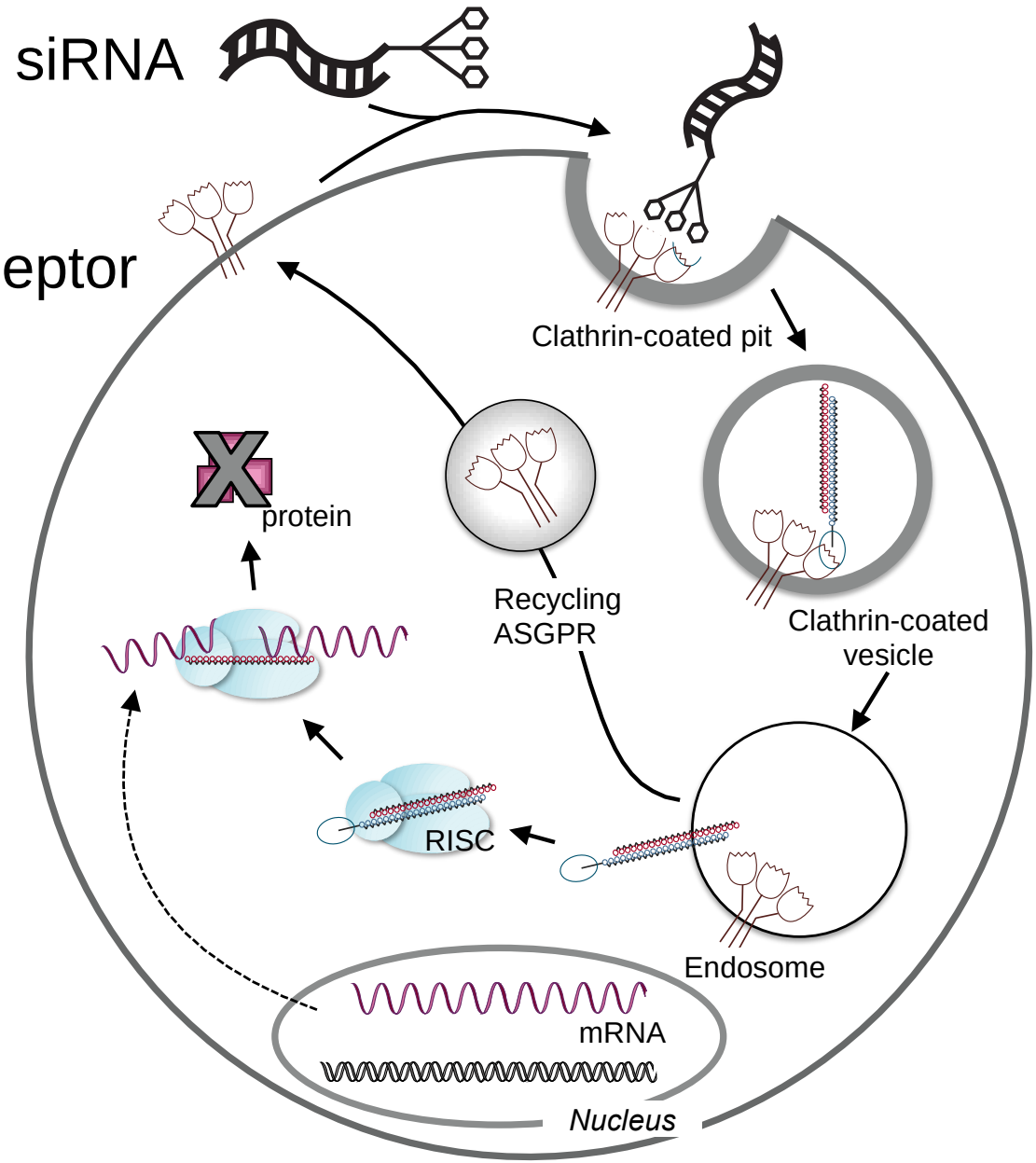
Lipid nanoparticle

Nanoparticle

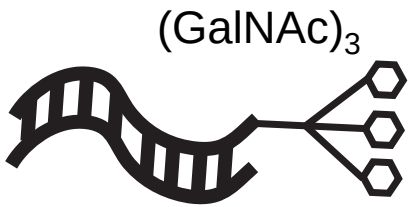
GalNAc-conjugates for liver targeting

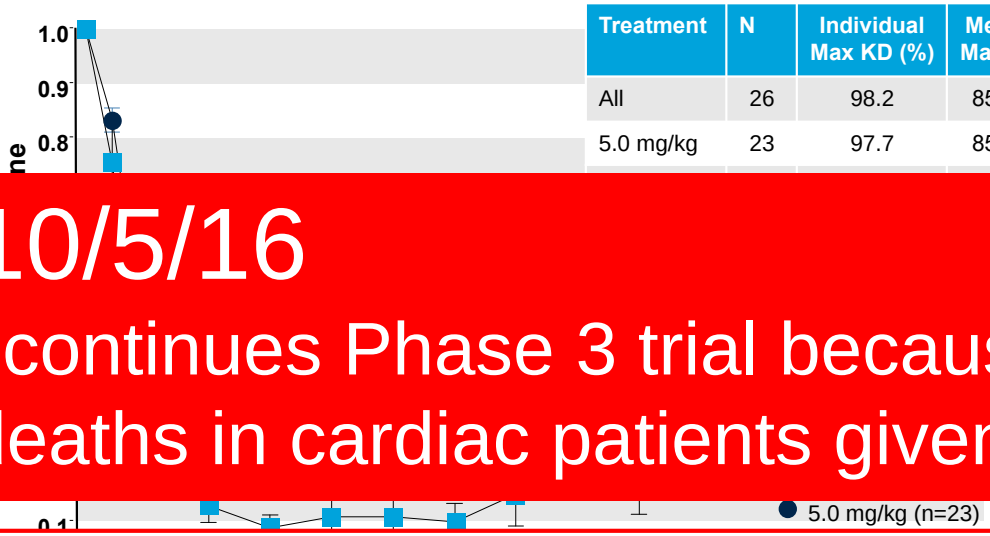
$(GalNAc)_3$-conjugated siRNA

Asialoglycoprotein receptor



GalNAc-conjugates for liver targeting





Treatment	N	Individual Max KD (%)	Mean ± SD Max KD (%)
All	26	98.2	85.9 ± 9.2
5.0 mg/kg	23	97.7	85.1 ± 9.3

Caveat 10/5/16

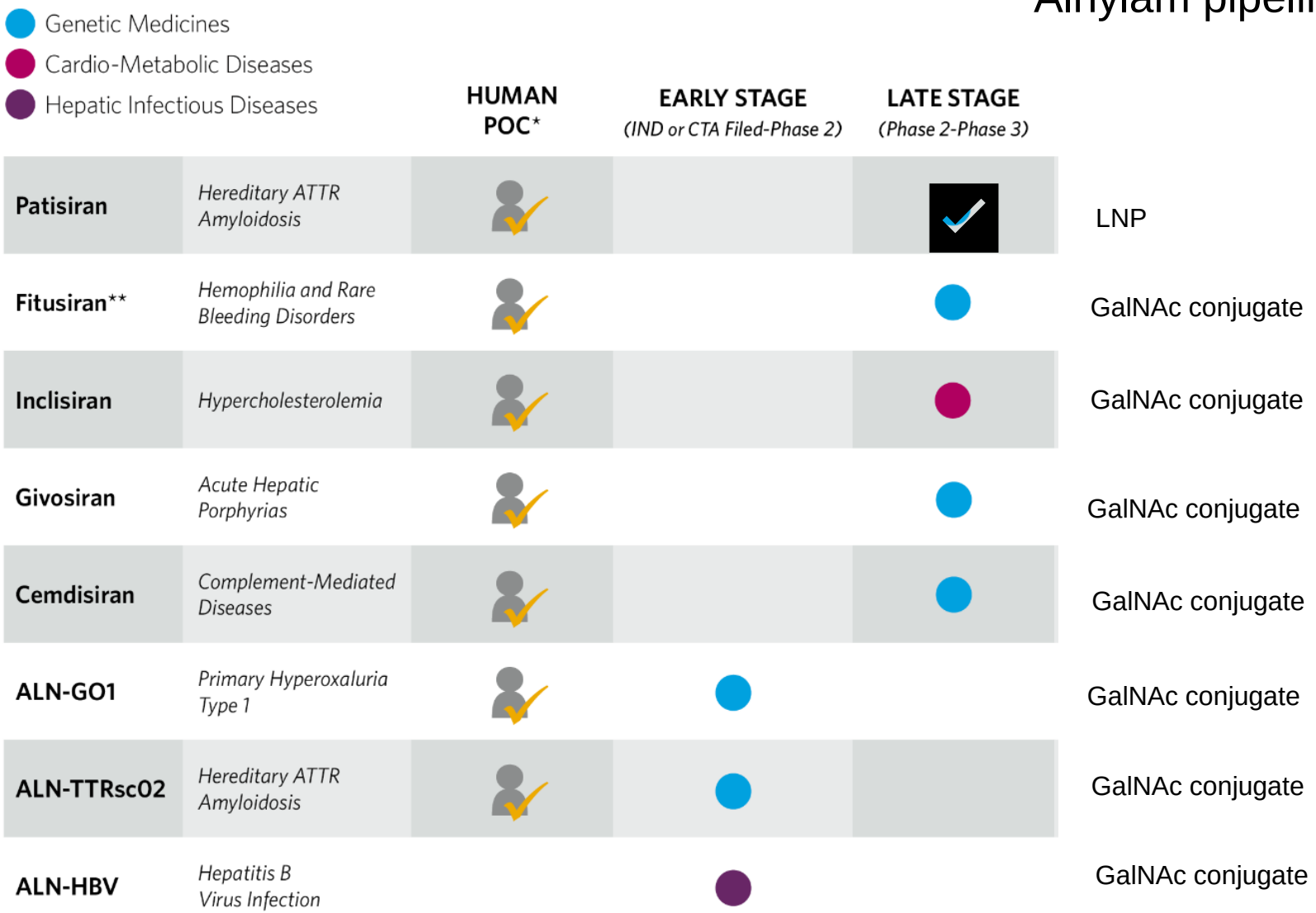
Alnylam discontinues Phase 3 trial because of increased deaths in cardiac patients given revusiran

Next generation chemically modified GalNAc-conjugates with enhanced stability and activity have 50x activity and require much lower doses

(from Alnylam press release 4/15)

siRNA drugs targeting the liver are rapidly advancing in the clinic

Alnylam pipeline

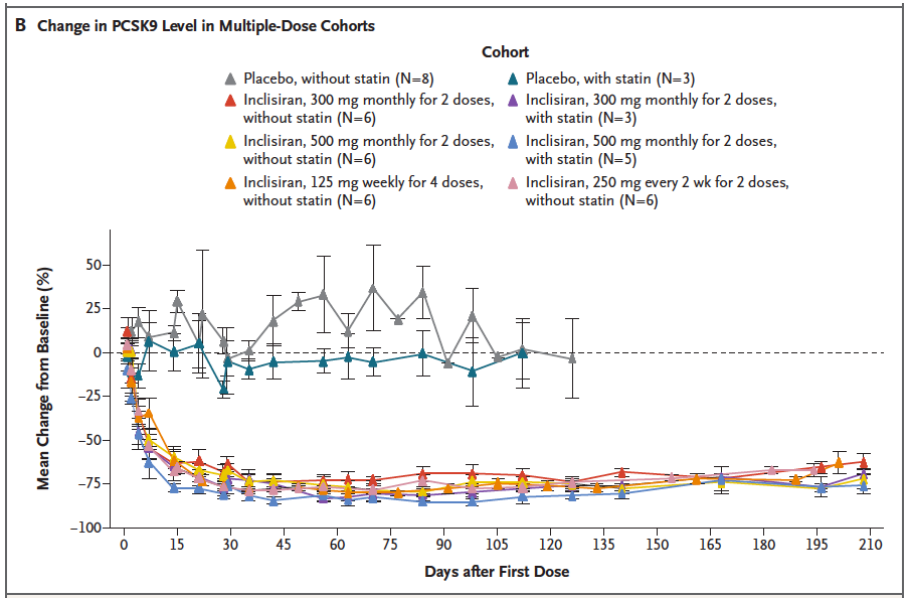


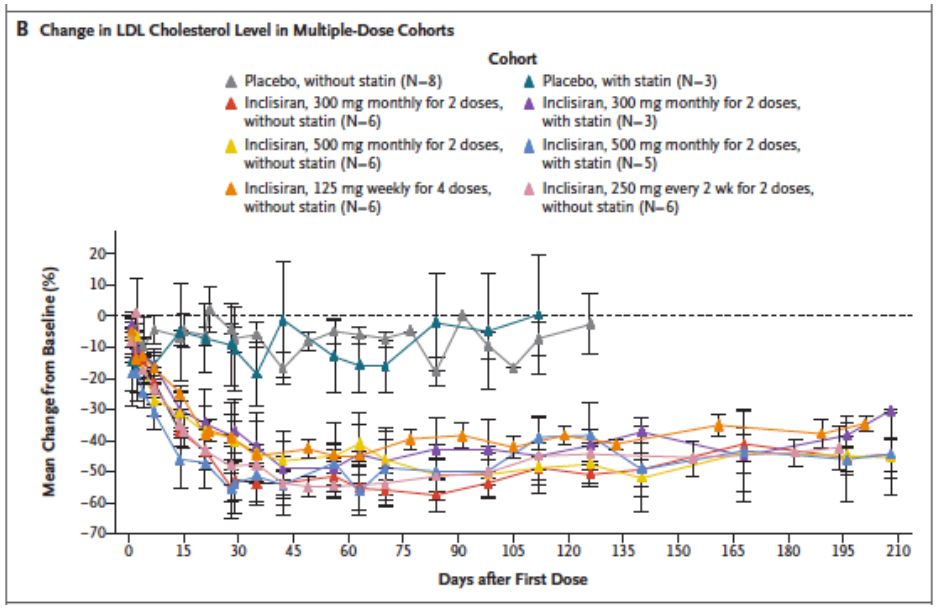
*Proof of concept (POC) defined as having demonstrated target gene knockdown and/or additional evidence of activity in clinical studies

The NEW ENGLAND JOURNAL of MEDICINE
N Engl J Med 2017;376:41-51.

ORIGINAL ARTICLE

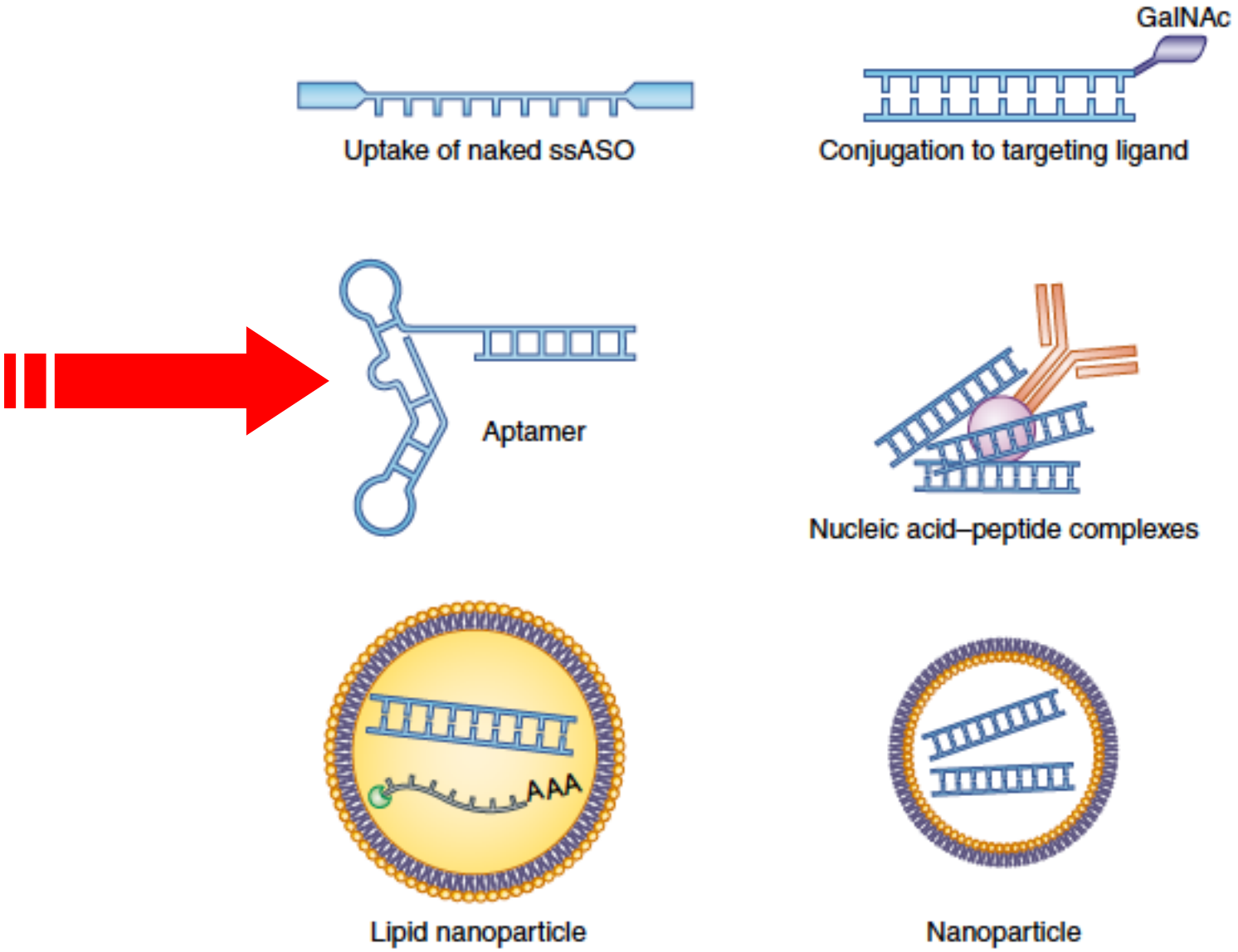
PCSK9 siRNA (inclisiran) to treat hypercholesterolemia uses enhanced chemistry and much lower drug exposure than revusiran



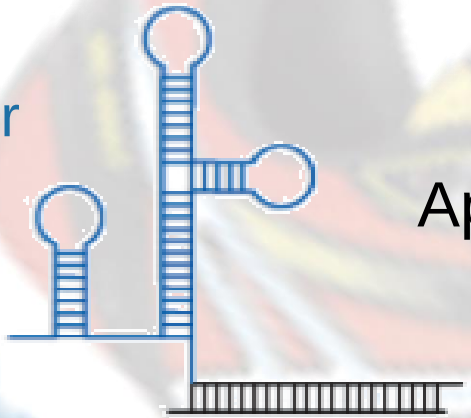


Well tolerated - only mild or moderate adverse events

siRNA delivery: beyond the liver?



Aptamer-siRNA chimeras (AsiCs) solve the problem of siRNA delivery beyond the liver



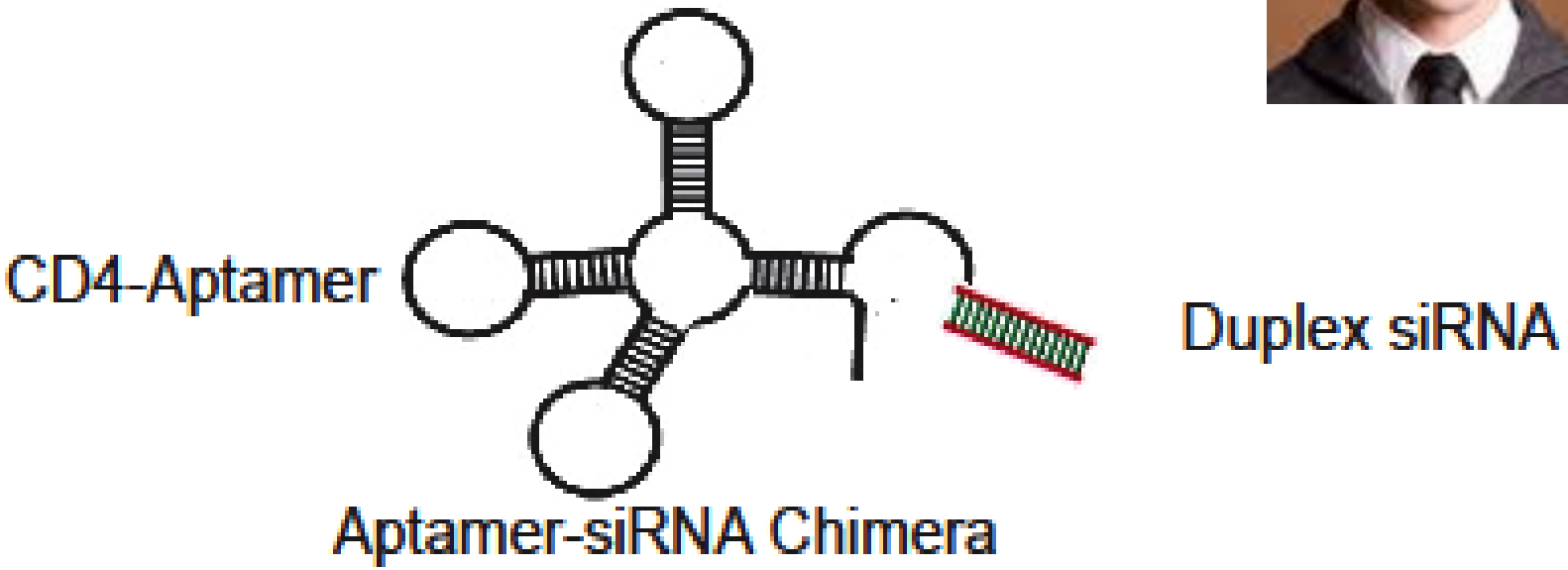
Aptamer-siRNA chimera (AsiC)

- One molecule - a targeting moiety (RNA aptamer) linked to an siRNA
- An aptamer is like an RNA antibody – it is selected to bind strongly and specifically to a single cell surface receptor
- An siRNA that selectively knocks down a gene only in targeted cells
- AsiCs are a flexible platform - by changing the aptamer, the target cell can be changed; the siRNA can be chosen to knockdown any gene

Platform developed by Paloma Giangrande and colleagues
McNamara et al., *Nature Biotech* 2006; Dassie et al., *Nature Biotech* 2009

CD4 aptamers deliver siRNAs to the immune cells that HIV infects and block HIV transmission

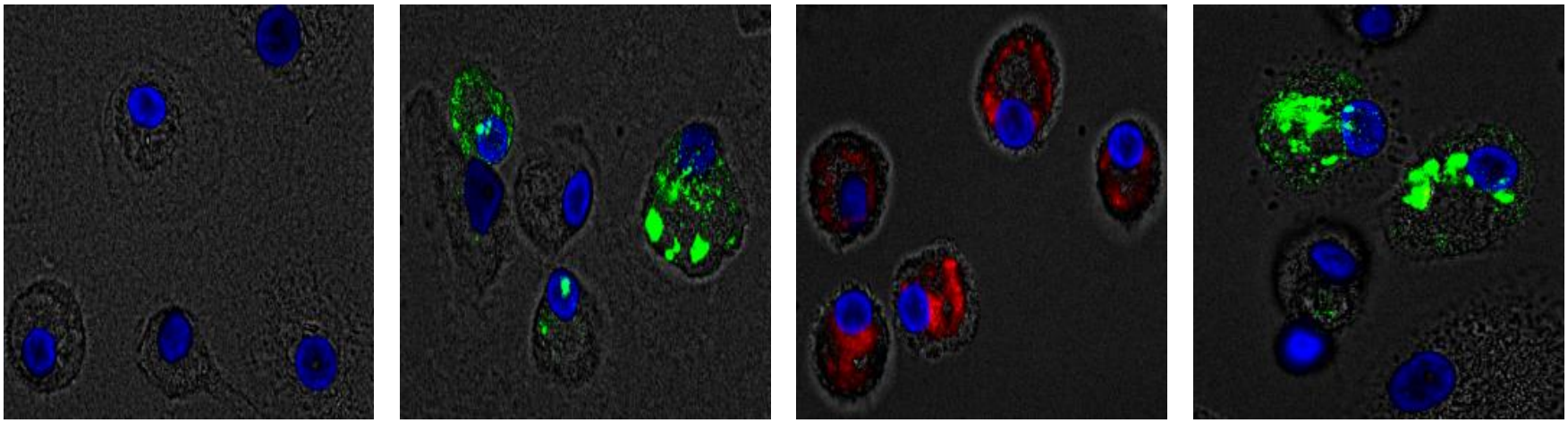
Lee Wheeler



Wheeler et al., J Clin Invest 2011; Mol Therapy 2013

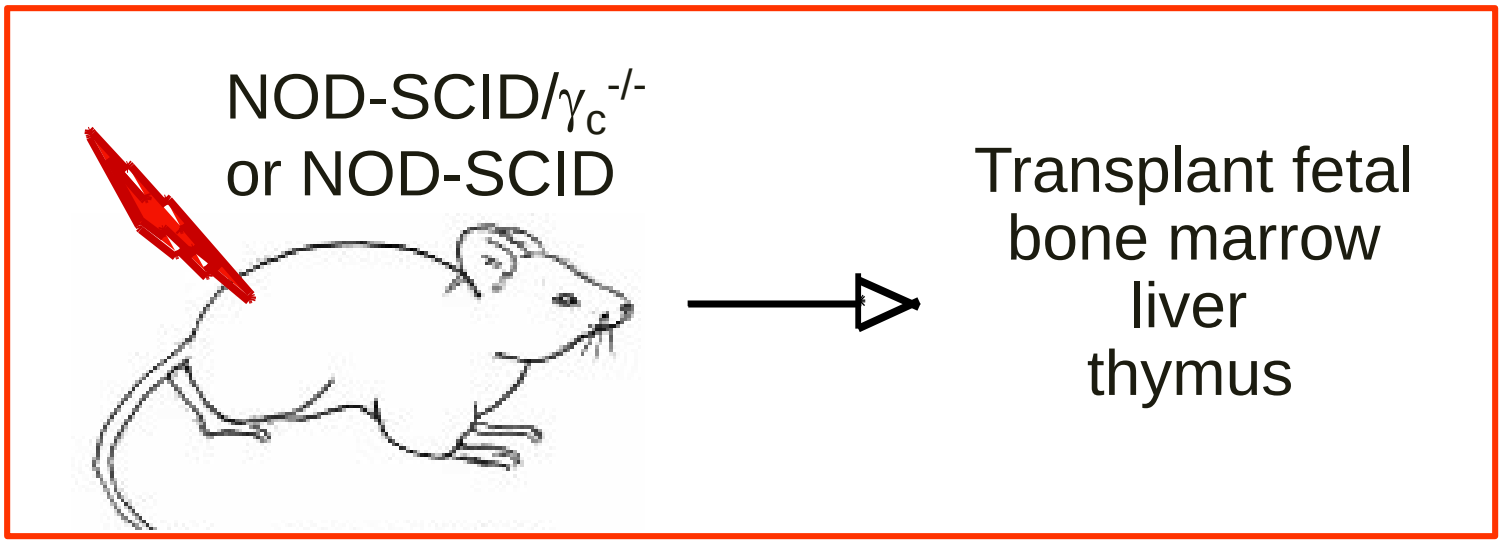
CD4 aptamer chimeras targeting *gag*, *vif* and *CCR5* inhibit HIV_{BaL} infection in monocyte-derived macrophages

Aptamer	-	-	CD4	PSMA
siRNA	-	-	gag, vif, Cy3-CCR5	gag, vif, Cy3-CCR5



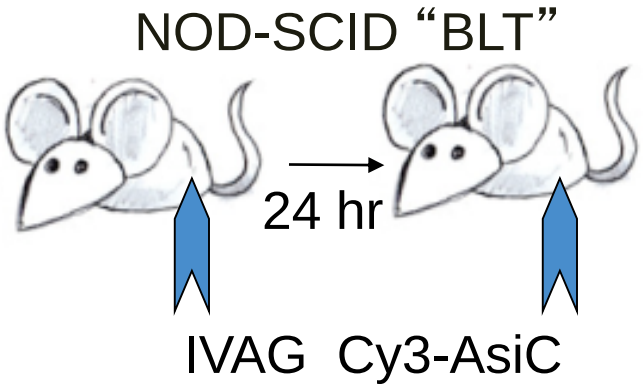
DAPI
HIV RNA by FISH
Cy3-siRNA

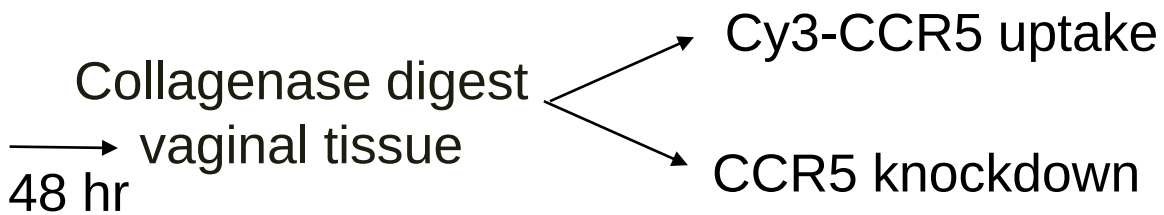
Testing protection in humanized mice



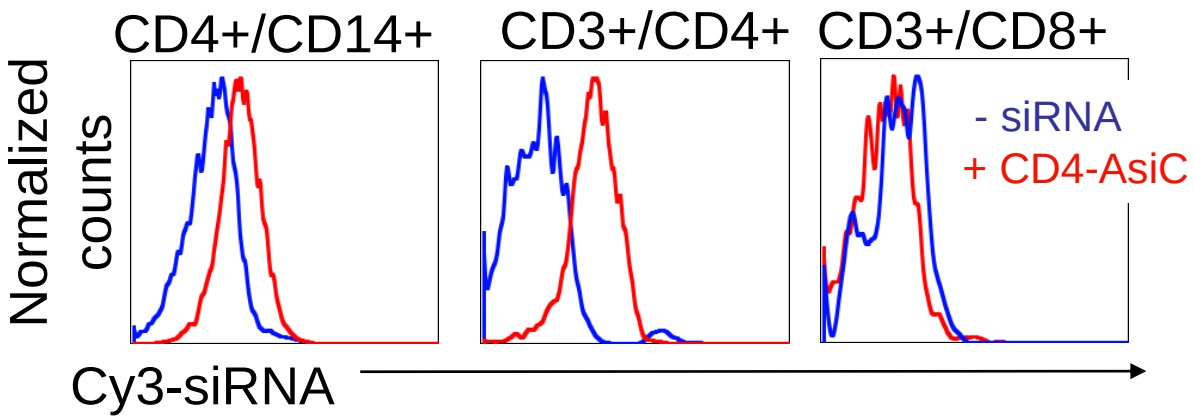
"BLT" mouse

Testing uptake and silencing in humanized mice

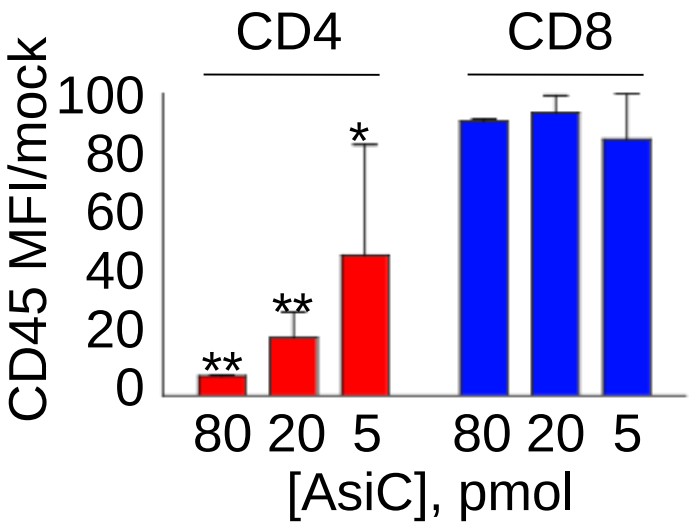


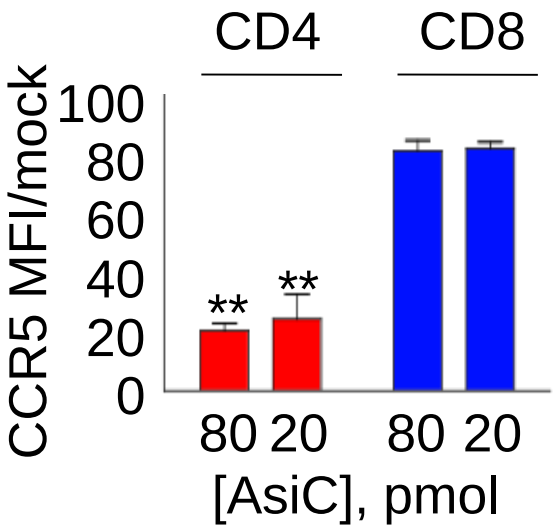


Uptake

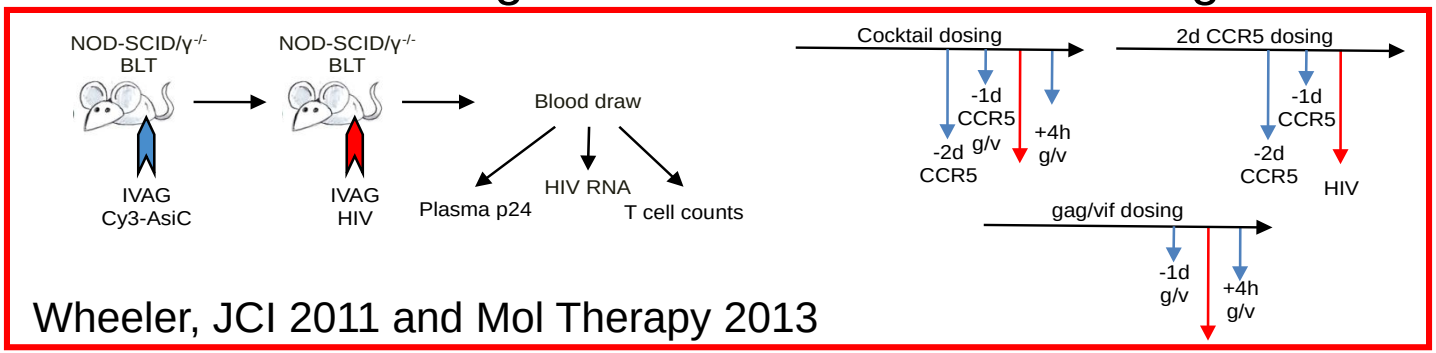


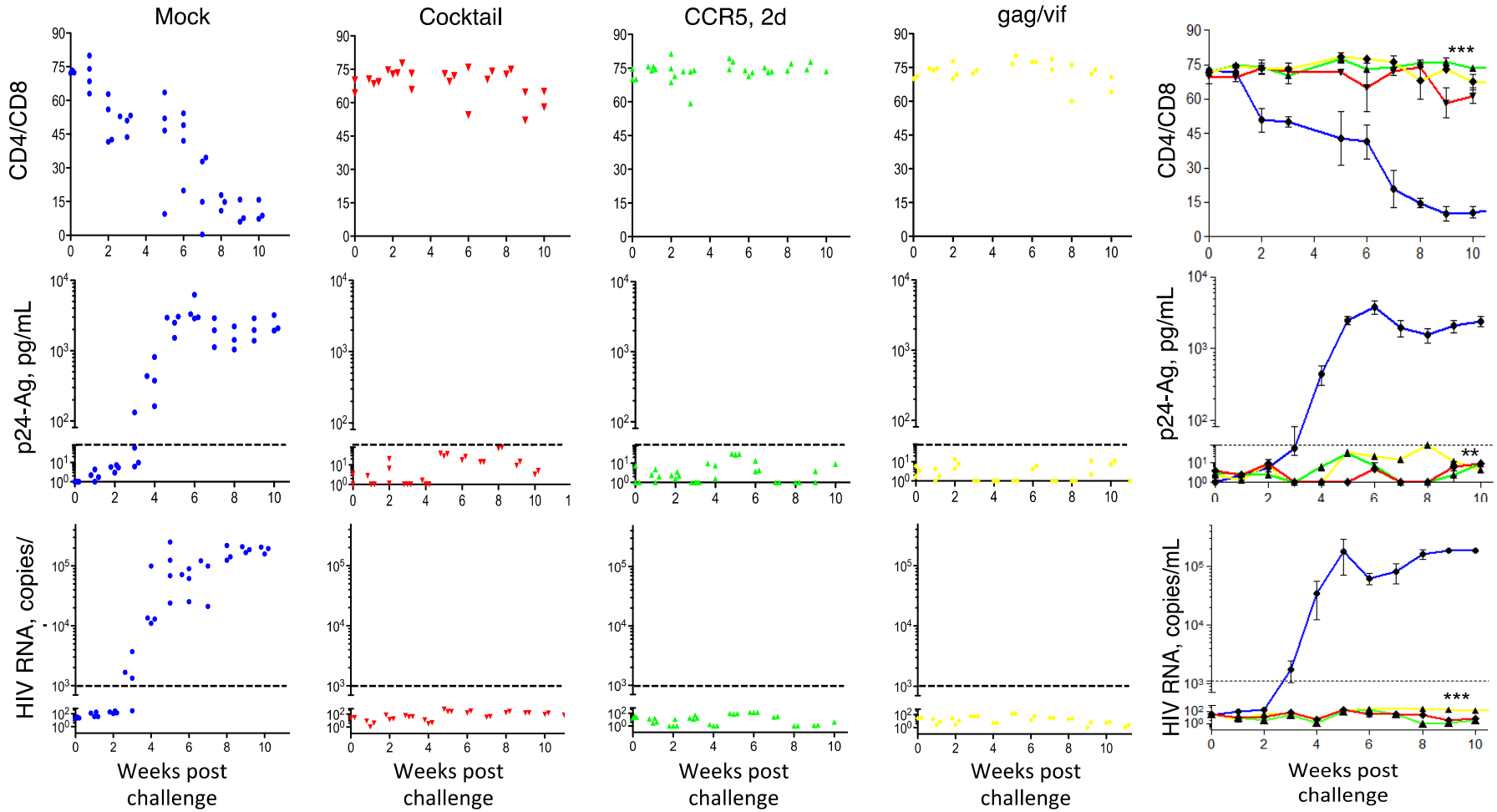
Knockdown





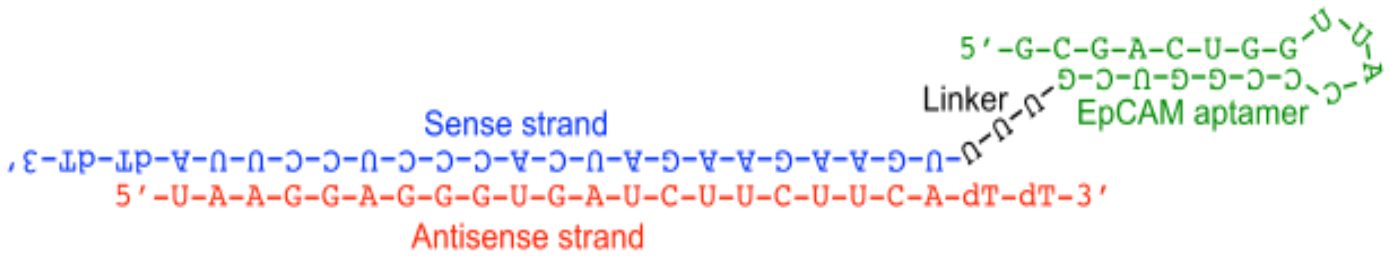
Protection from HIV challenge with CD4-AsiCs directed against CCR5 or HIV





Targeting Epithelial Cancers and their Stem Cells with EpCAM AsiCs

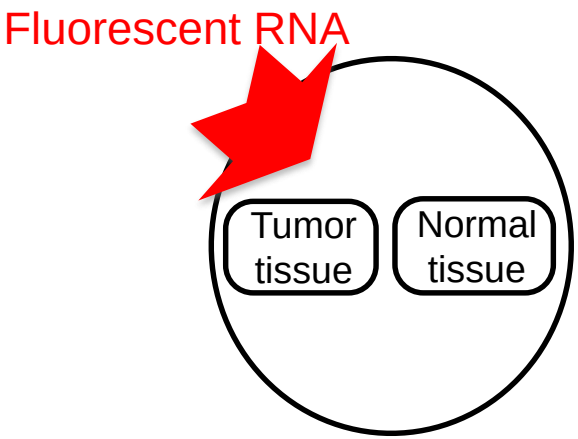
- EpCAM expressed at gap junctions at low levels on normal epithelial cells, but much more highly (100-1000-fold greater) throughout the membrane of all epithelial cancers
- EpCAM is a marker of epithelial cancer stem cells – cells responsible for relapse and metastasis
- As proof-of-concept, the siRNA targets *PLK1*, a kinase required for mitosis
- 19 nt EpCAM aptamer (Shigdar and Duan, 2011 Cancer Sci)



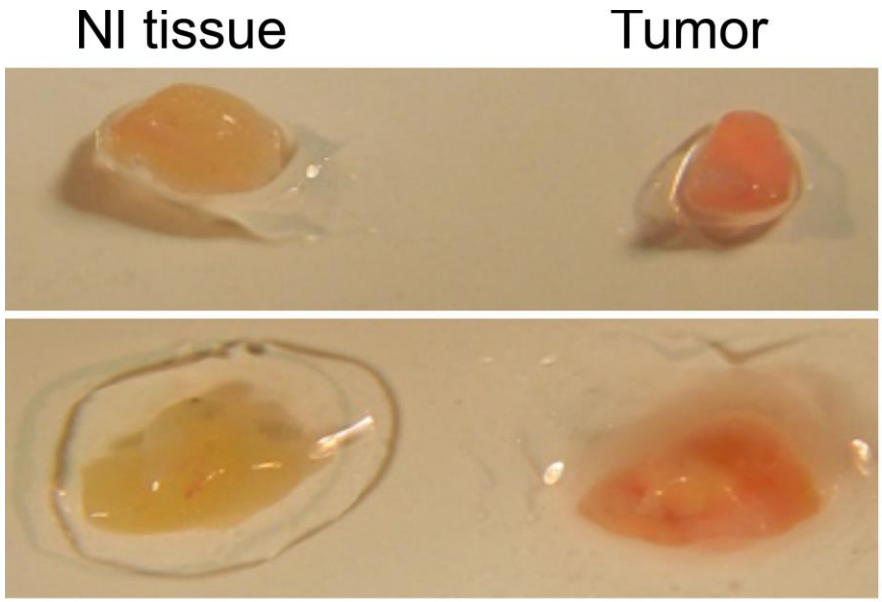
Adi Gilboa-Geffen

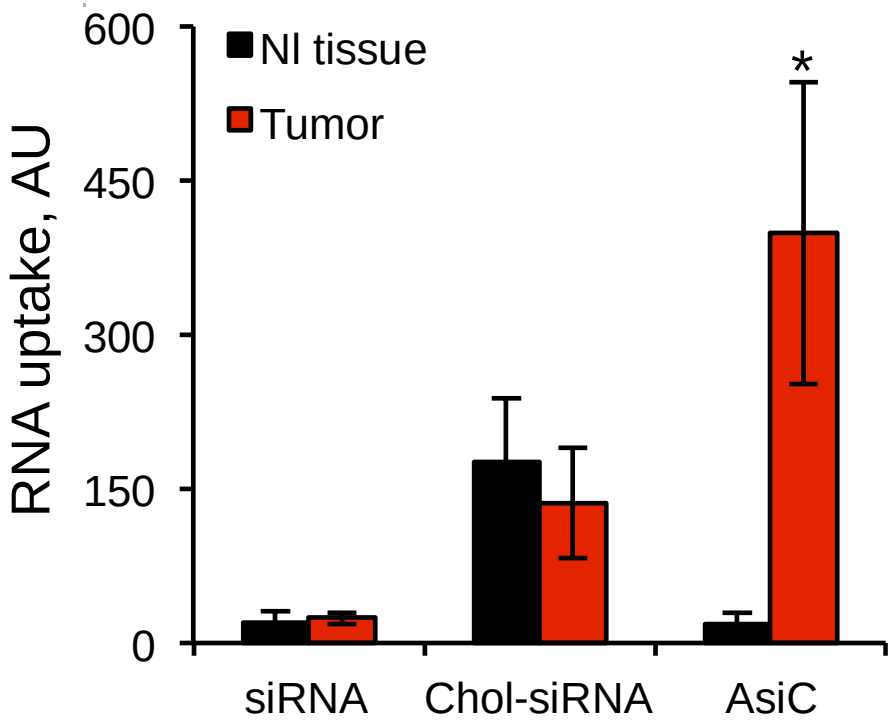
(Mol Cancer Ther 2015)

Human TNBC tumors take up Cy3-EpCAM-AsiC compared to normal breast tissue

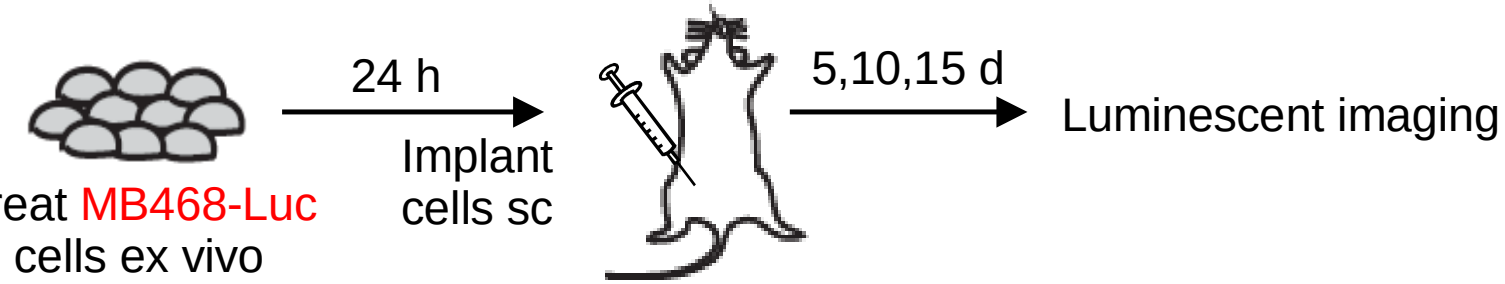


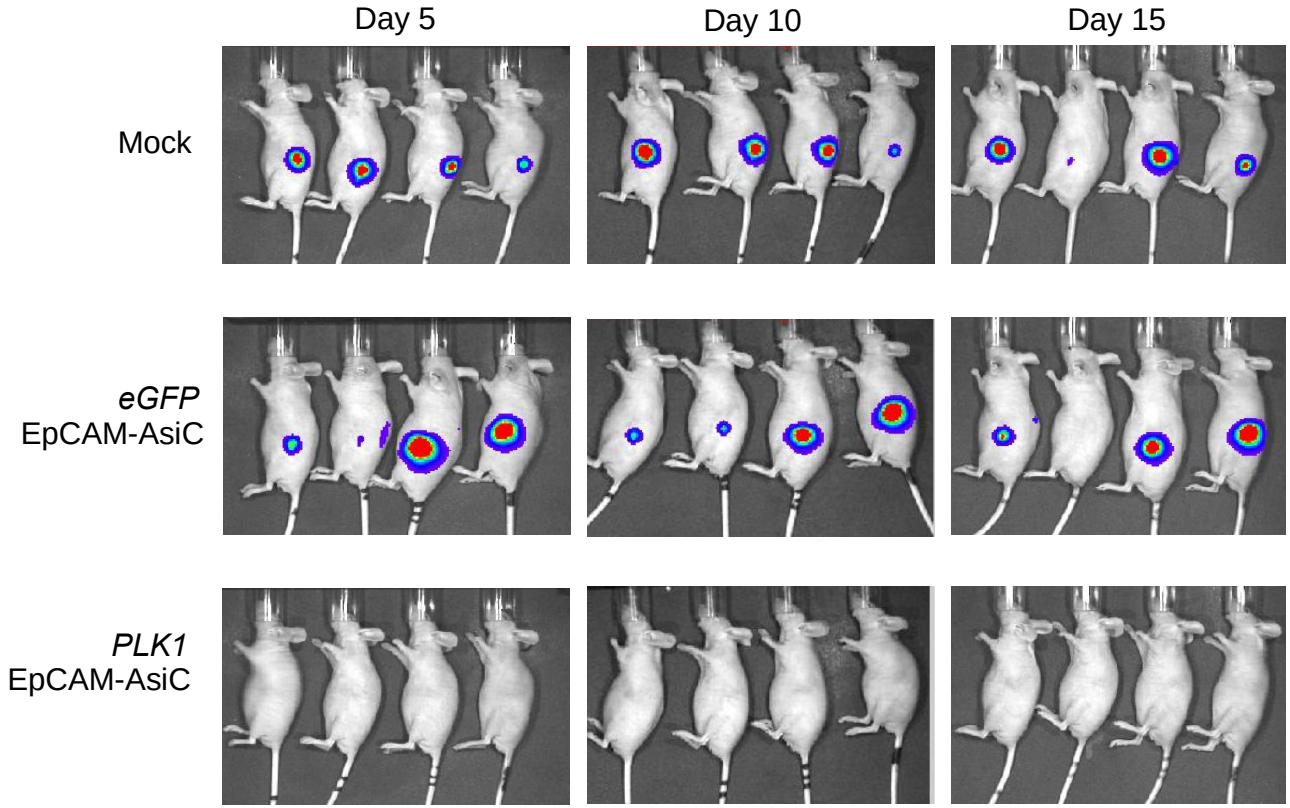
Analyze fluorescent RNA uptake after 3 d by FACS

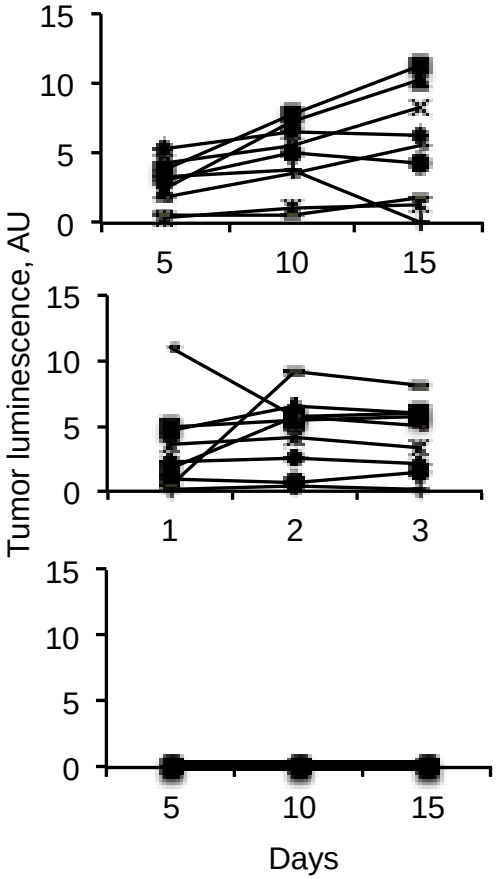




Ex vivo treatment of EpCAM+ TNBC cells prevents tumor initiation

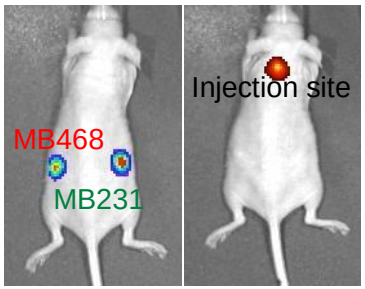




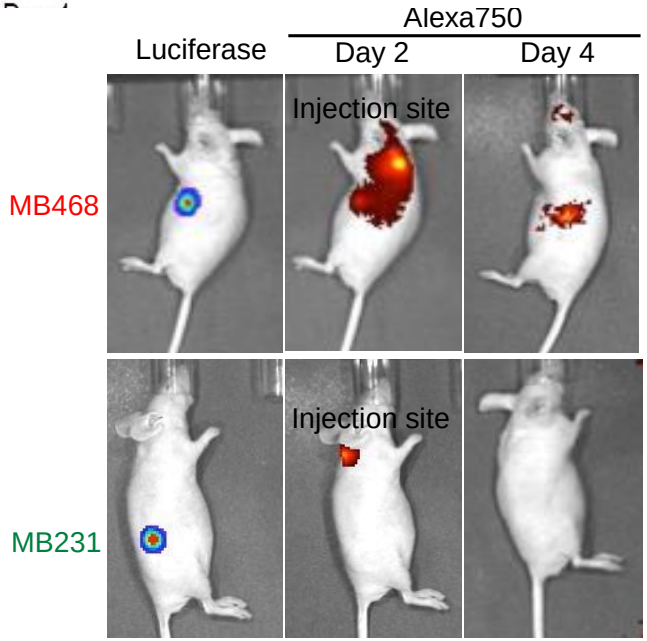


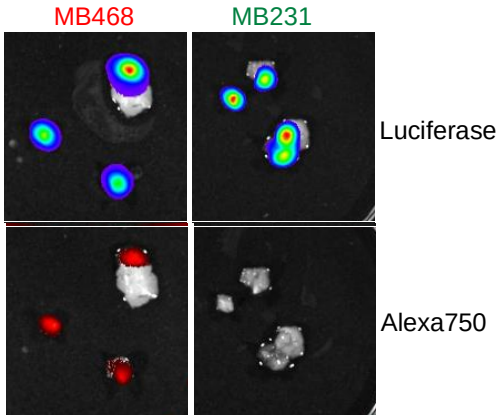
Selective uptake of Alexa750-EpCAM-AsiCs into EpCAM+ tumors

15 min post injection

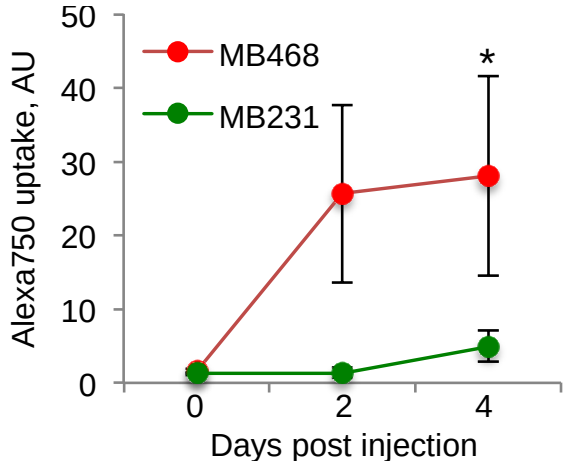


N=8 mice



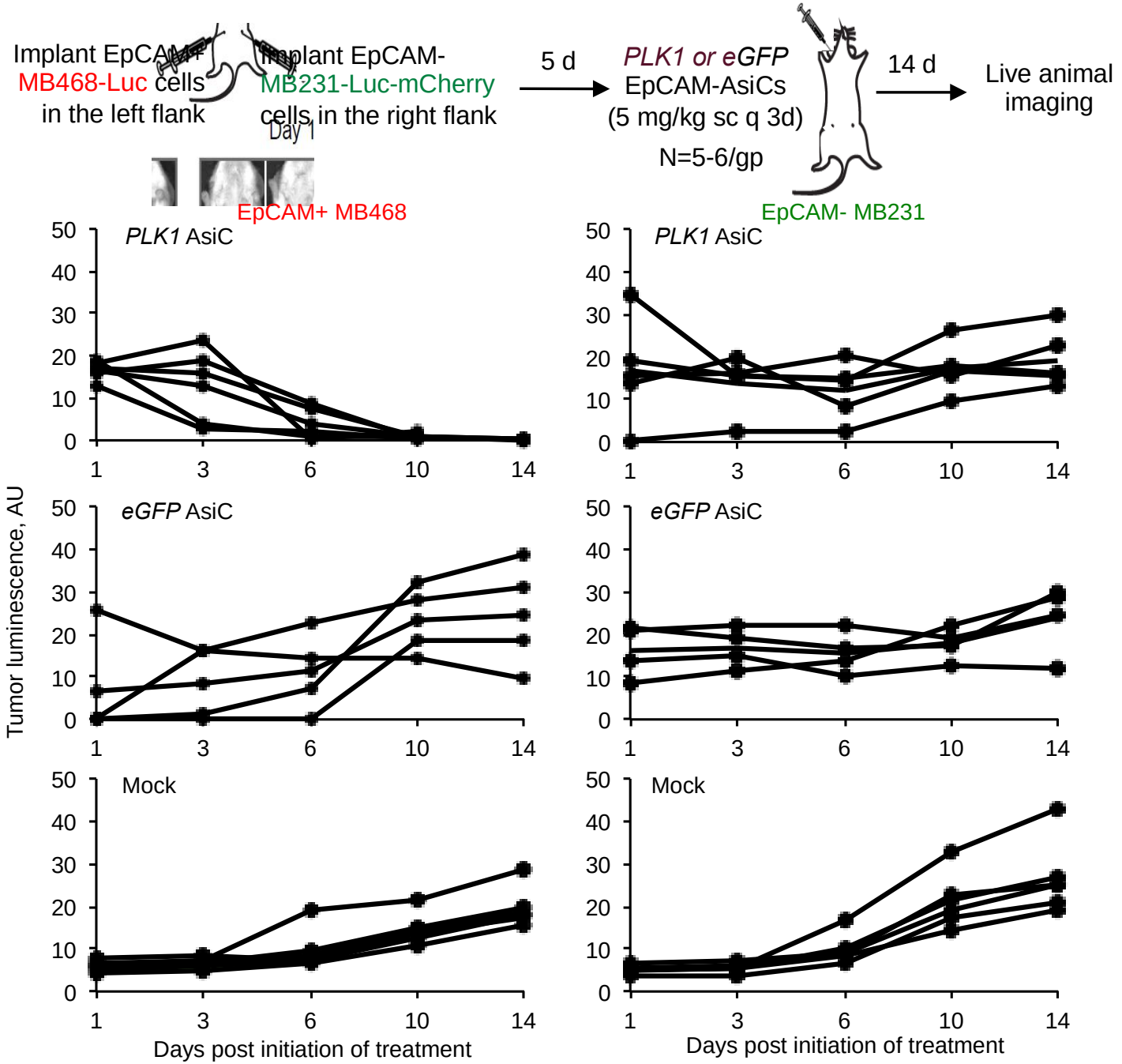


siRNA uptake by tumor



Excised tumors

EpCAM-AsiCs targeting *PLK1* inhibit EpCAM+ TNBC tumor growth



Summary

EpCAM-AsiCs knockdown genes in epithelial breast cancer cells and the tumor-initiating cells within them, sparing normal epithelial cells

Subcutaneously injected EpCAM-AsiCs localize to distant tumors

PLK1 EpCAM-AsiCs suppress tumor growth in vitro and in vivo

PLK1 EpCAM-AsiCs eliminate tumor-initiating cells

AsiCs do not trigger innate immunity

Most common epithelial tumors are EpCAM+ (colon, lung, prostate, pancreas). Similar results in HCT116 colon cancer xenografts. This may be a good platform for targeting other cancers.

Will small RNAs that harness RNAi become the next new class of drugs?

Prospects look very good…