

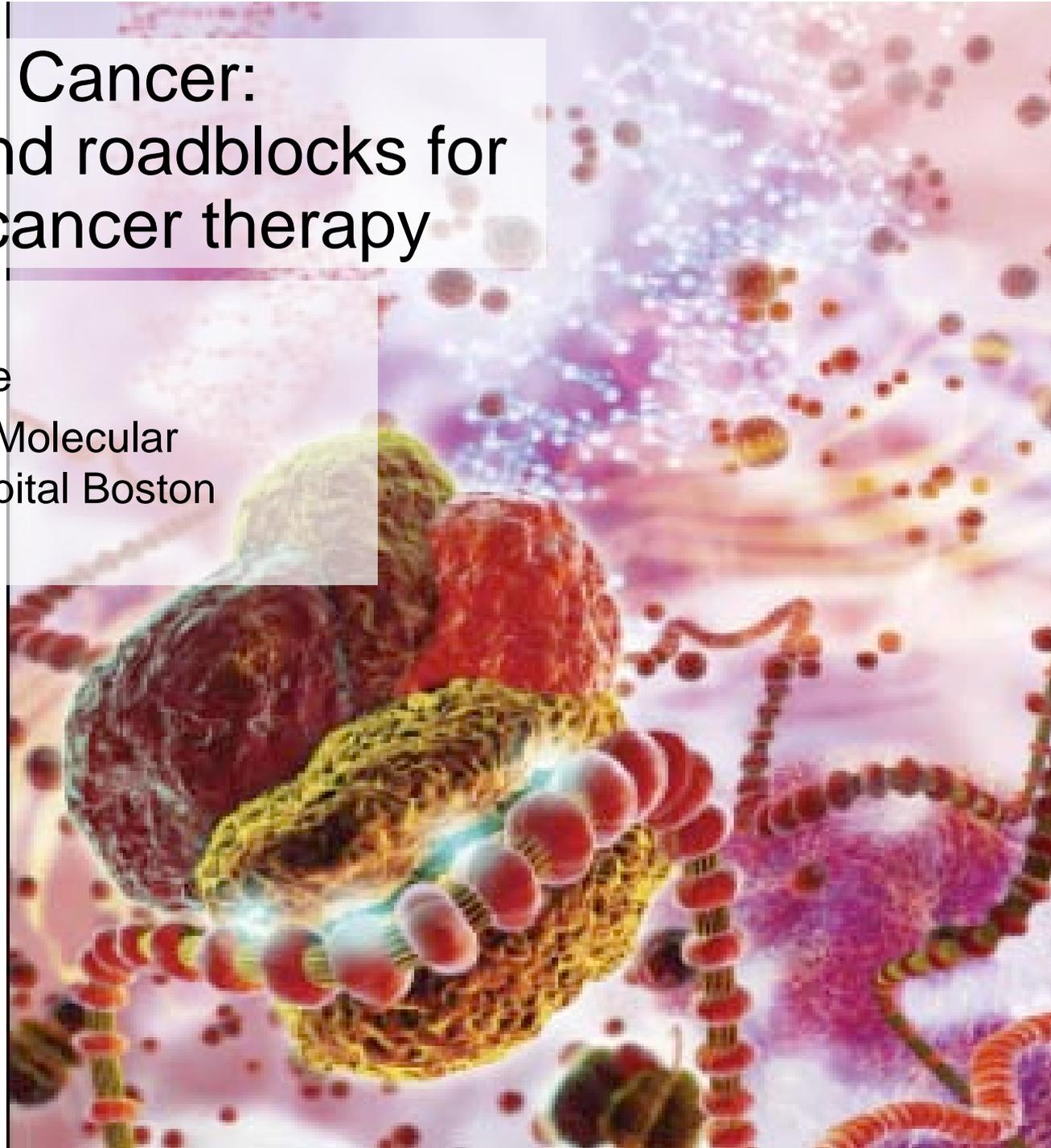
Micromanaging Cancer: opportunities and roadblocks for miRNA-based cancer therapy

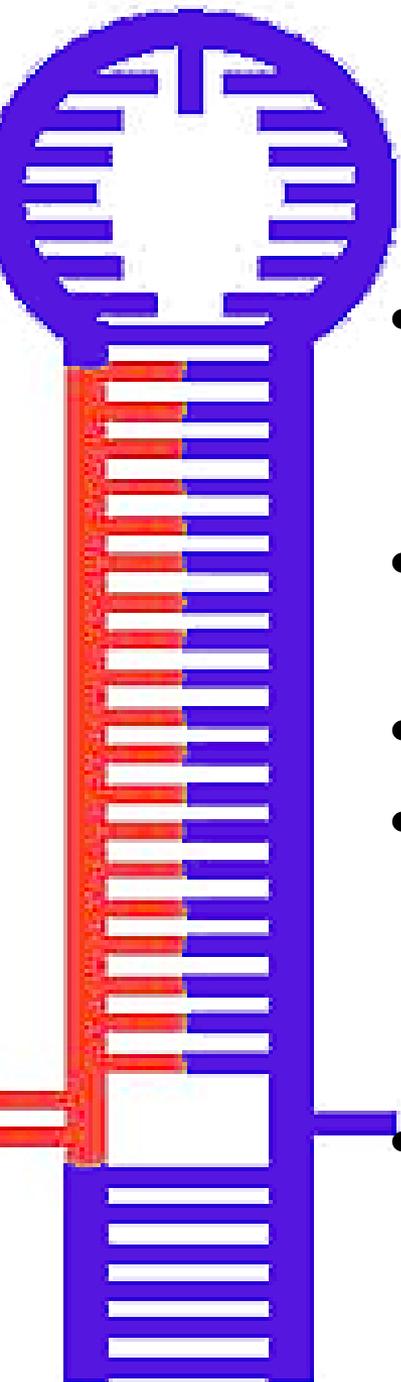
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Rationale for manipulating microRNA expression to treat cancer

- microRNAs orchestrate a cell's response to environmental or developmental cues by modulating the expression of hundreds of genes
- microRNA expression is the best indicator of a cell's state of differentiation
- miRNA mis-expression common in cancer
- by antagonizing or mimicking a microRNA, it may be possible to alter cell survival, proliferation, differentiation state, metastatic capability, sensitivity to chemo or radiation
- because microRNAs regulate many genes, the therapeutic effect could be more profound than targeting one gene at a time and the likelihood of escape mutation may be low

Some of the most studied oncomirs

 Tumor suppressor
 Oncogene

miRNA	Biologic Process	Type of Cancer	Key Target
<i>let-7</i>	Cell cycle, differentiation	Lung, breast	<i>LIN28, hRAS, KRAS, HMGA2, TRIM71, NF2</i>
<i>miR-15/16</i>	Apoptosis	CLL, prostate	<i>BCL2, PDCD4, MCL1, RAB21, ACTR1A, JUN, PRIM1</i>
<i>miR-17/20/93/106</i>	Cell cycle, apoptosis, angiogenesis, senescence	B-cell lymphoma/leukemia, lung, stomach, colon, pancreas, prostate, thyroid, neuroendocrine, neuroblastoma	<i>E2F1, CDKN1A, NCOA3, RUNX1, VEGFA</i>
<i>miR-19</i>	Cell cycle, apoptosis	B-cell lymphoma/leukemia	<i>PTEN</i>
<i>miR-21</i>	Cell cycle, apoptosis	Breast, colon, lung, pancreas, prostate, stomach, biliary, brain, liver, multiple myeloma	<i>CDK6, PDCD4, CDKN1A, FAS, IL6R, SOCS5, APAF1, NFIB, TPM1</i>
<i>miR-24</i>	Cell cycle, differentiation	Colon, pancreas, CLL	<i>E2F2, MYC</i>
<i>miR-25/92</i>	Cell cycle, apoptosis, angiogenesis	B-cell lymphoma/leukemia, lung, stomach, colon, pancreas, prostate, thyroid, neuroendocrine, neuroblastoma	<i>BCL2, L11, CDKN1C</i>
<i>miR-26</i>	Cell cycle	Liver	<i>CCND2, CCNE2</i>
<i>miR-34</i>	Cell cycle, apoptosis, DNA repair, differentiation	Liver, prostate, breast, lung, neuroblastoma	<i>NOTCH1, BCL2, E2F3, MYCN, DLL1, VEGFA, CCND1, CDK6, SIRT1</i>
<i>miR-141/200</i>	Differentiation, metastasis	Ovarian, prostate, kidney	<i>ZEB1, ZEB2</i>
<i>miR-155</i>	Cell cycle, differentiation	B-cell lymphoma/leukemia, lung, breast, nasopharyngeal	<i>BACH1, AGTR1, LDOC1, MATR3, TM6SF1, AGTR1, AID, SHIP1</i>
<i>miR-221/222</i>	Cell cycle	Thyroid, pancreas, stomach, prostate, melanoma	<i>CDKN1B, CDKN1C, KIT</i>

Cancer-related miRNAs (oncomirs) can act as oncogenes or tumor suppressors

Antagonizing oncogenic miRNAs vs mimicking tumor suppressor miRNAs for cancer therapy?

- Antisense delivery is easier (single-strand vs double strand oligo)
- However, reduced miRNA expression is a hallmark of poorly differentiated cells and cancer
 - Global reduction of mature miRNAs in cancer, especially poorly differentiated
 - Down-regulation or mutation of Dicer and Drosha or miRNA processing genes associated with cancer and poor survival
 - miRNA processing (i.e. lin28/let-7) suppressed in poorly differentiated cancers

If delivery were not an issue, miRNA replacement therapy would make the most sense.

Where we stand: Phase I siRNA trials for cancer

- Early results reasonably encouraging
- toxicity manageable but could be improved
 - some evidence of delivery and gene silencing but delivery still suboptimal
 - stable disease in some treated patients
 - one major response (endometrial ca)



(Alynlam 6/11)



*Response ongoing after 24 doses

Attractive novel features of siRNA and miRNA therapy

- Opens up a universe of non-“druggable” targets
- Rapid drug development – once a target is chosen, clinical testing possible within ~1-2 years
- Drug efflux/multidrug resistance unlikely to be an issue
- Ideal platform for personalized therapies using siRNA/miRNA cocktails
- Relatively prolonged intracellular activity (~5 d for rapidly dividing cells) compared to small molecule drugs
- May be able to target cell populations resistant to conventional drugs

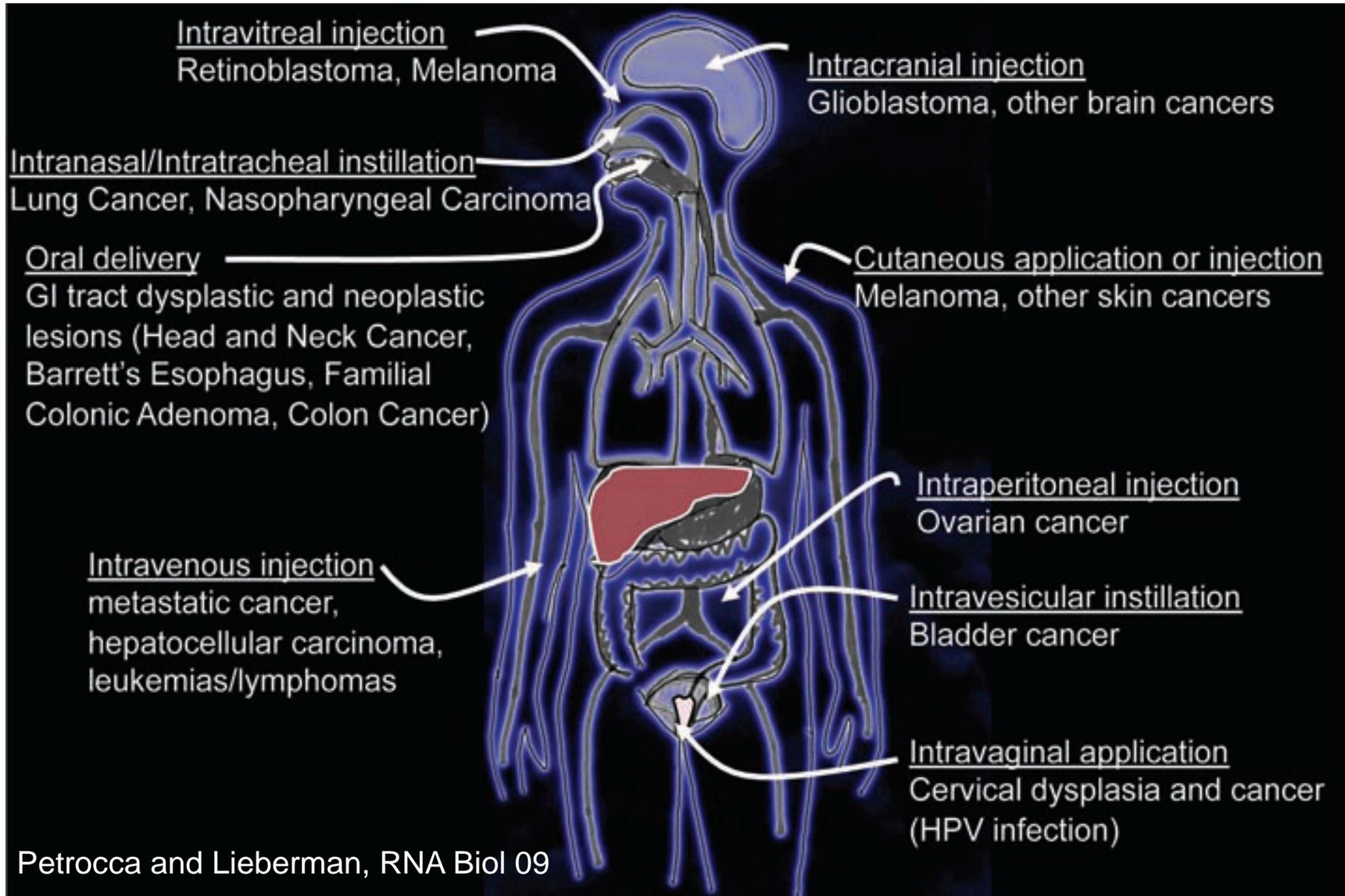
Key shared hurdles for siRNA/miRNA therapy

- **Intracellular systemic delivery in vivo – uptake into cell and trafficking within cell to cytosol**
- Off target effects – knockdown of unintended targets/immune stimulation
- Toxicity to normal dividing cells
- Half-life

siRNA vs miRNA therapeutics

siRNA	miRNA
Single-gene silencing	Targeting a gene network (will include anti-oncogenic and pro-oncogenic genes) – less control?
More potent knockdown of an individual gene	Less potent knockdown of individual genes
Unlikely to compete with endogenous miRNA pathway	More likely to compete with endogenous processes?
Tumor escape mutation more likely	Tumor escape less likely
Need double-stranded oligo	Possible to antagonize with single stranded ASO
Can only suppress one or a few genes	Could restore tumor suppressor miRNAs to cancer cells that lack them
More predictable	Less predictable effect

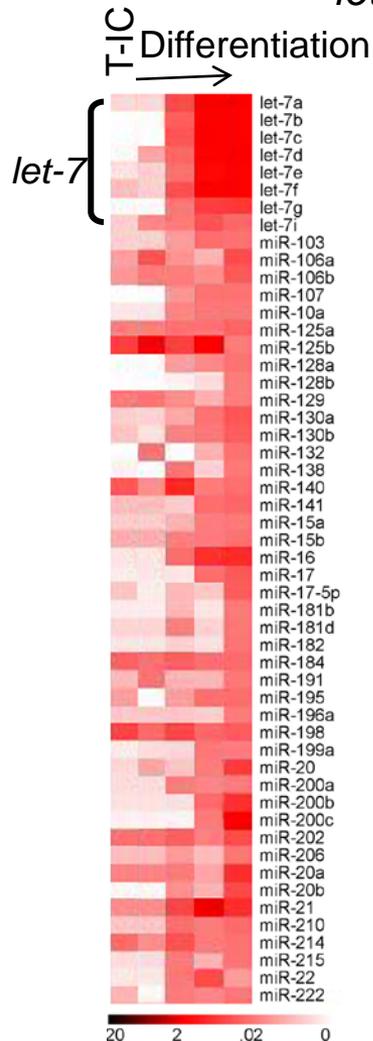
Since systemic delivery is a problem, are there clinical indications for more localized therapy?



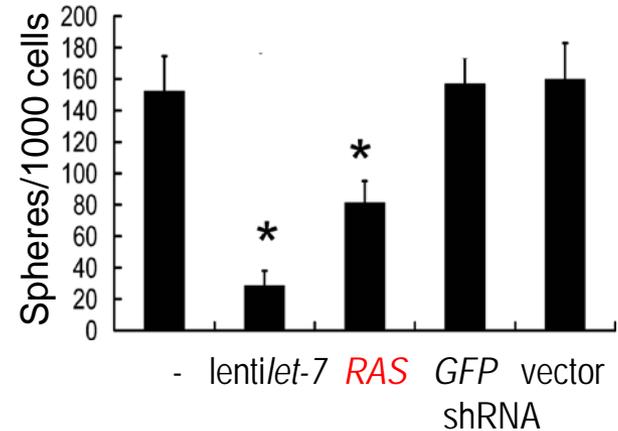
Tumor-initiating cells (“cancer stem cells”) are resistant to drug therapy and may be responsible for recurrence and metastasis

Could miRNAs be used to differentiate or kill these cells?

let-7 is not expressed in breast T-IC



Expressing *let-7* or silencing its target *RAS* leads to loss of self-renewal



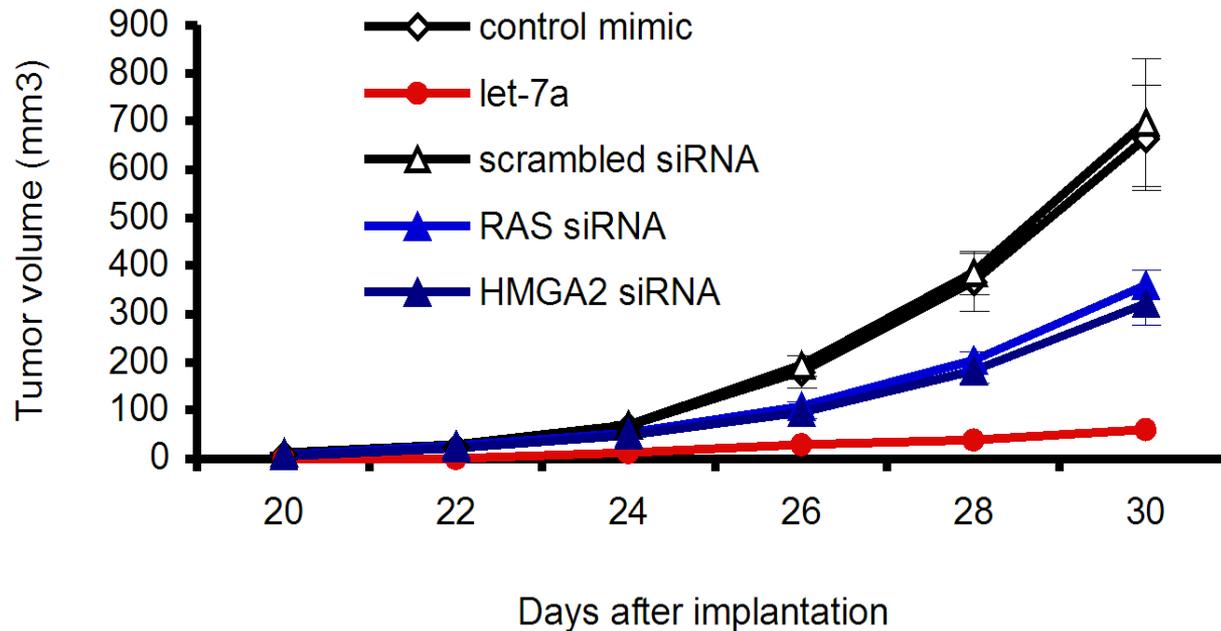
let-7-expressing breast T-IC are less tumorigenic and less likely to metastasize

of cells implanted

Transduced	2x10 ³ cells			2x10 ⁴ cells			2x10 ⁵ cells		
	Tumor	Lung met	Liver met	Tumor	Lung met	Liver met	Tumor	Lung met	Liver met
-	8	6	3	10	7	4	10	8	6
vector	8	5	3	10	8	5	10	8	5
<i>let-7</i>	2	1	0	5	3	1	7	4	3

(10 mice/group)

Intratatumoral injection of *let-7*-containing inhibits breast T-IC tumors more effectively than knocking down individual *let-7* regulated target genes



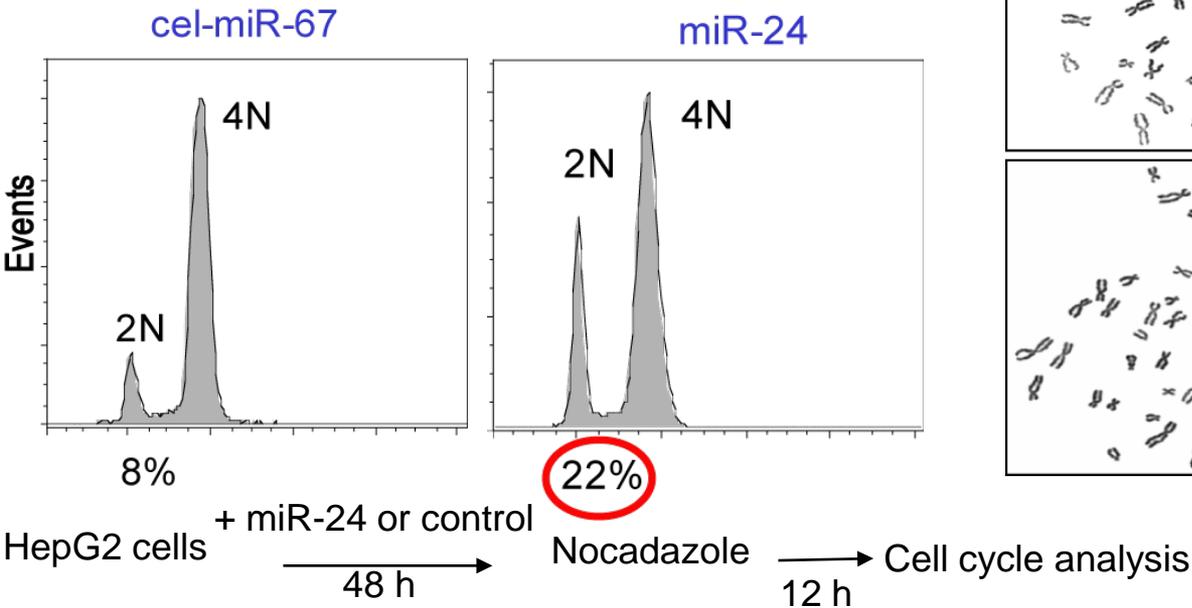
2×10^5 SK-3rd cells implanted into mammary fat pad.

From D2, 2 mg/kg siRNA injected 3 times a week in liposomes.

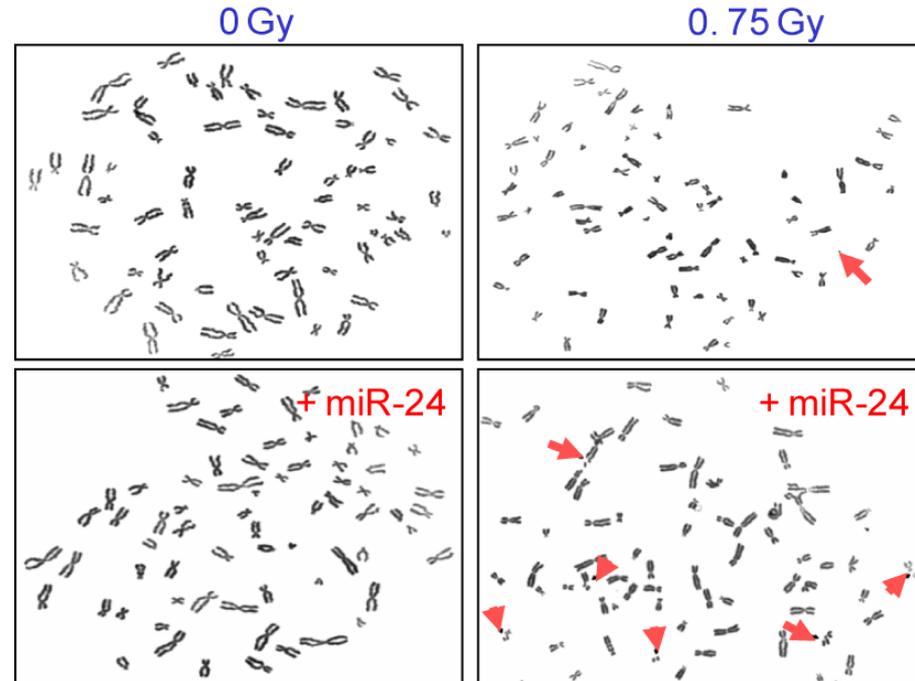
miR-24: inhibitor of cell division and DNA repair

A perfect anti-cancer drug?

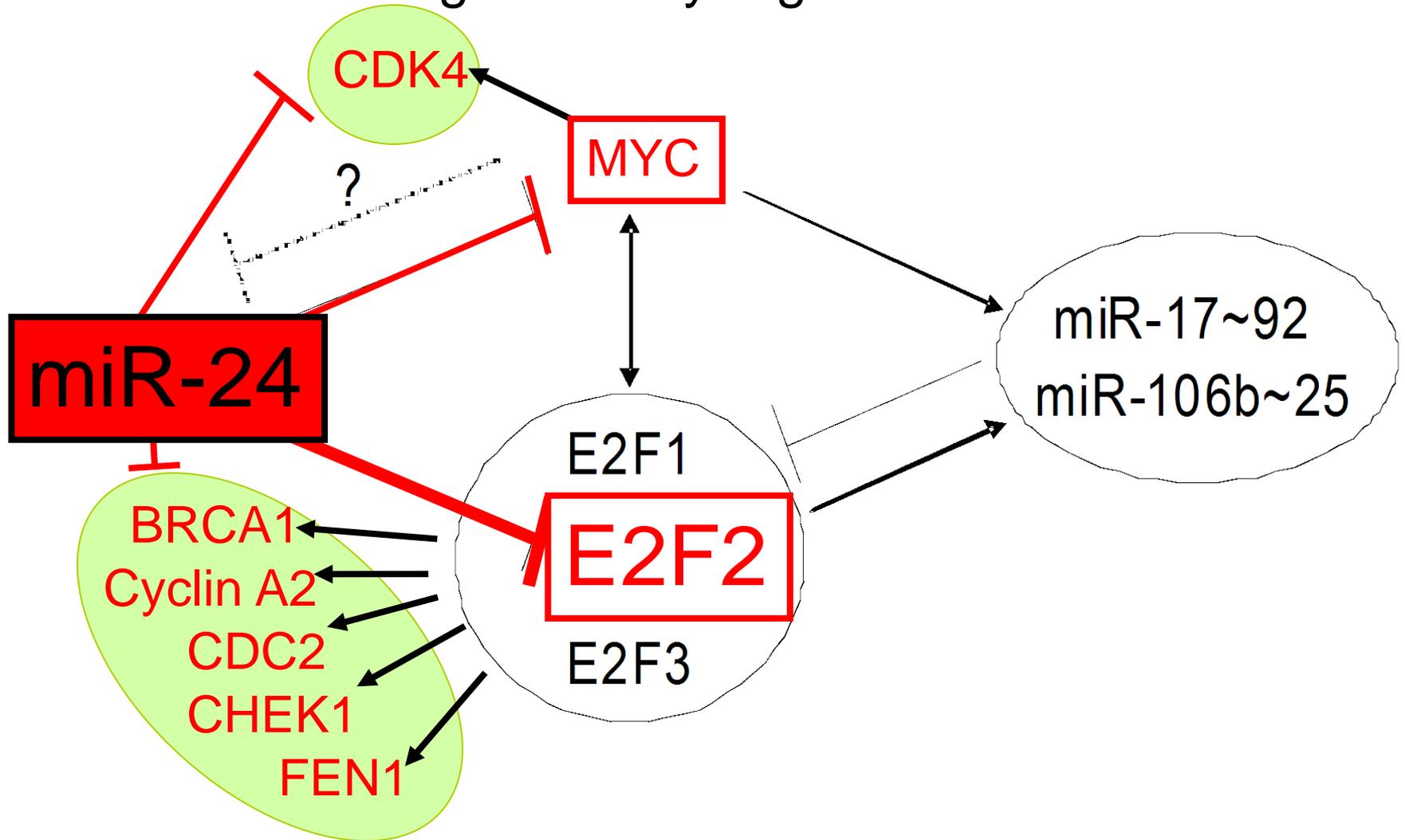
miR-24 mimics cause G1/S arrest



miR-24 sensitizes K562 cells to DNA damage



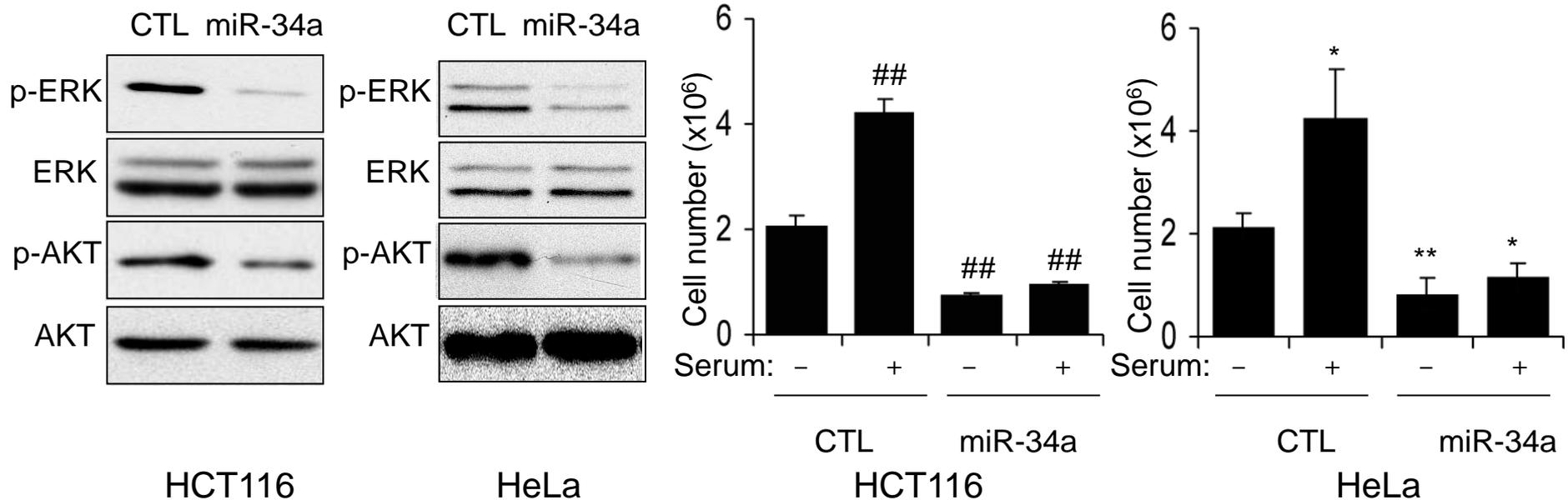
miR-24 counteracts the oncogenic miR-17~92 cluster.
miR-24 directly regulates MYC and E2F2 and some of the genes they regulate.



The miR-34a: another tumor suppressor miRNA

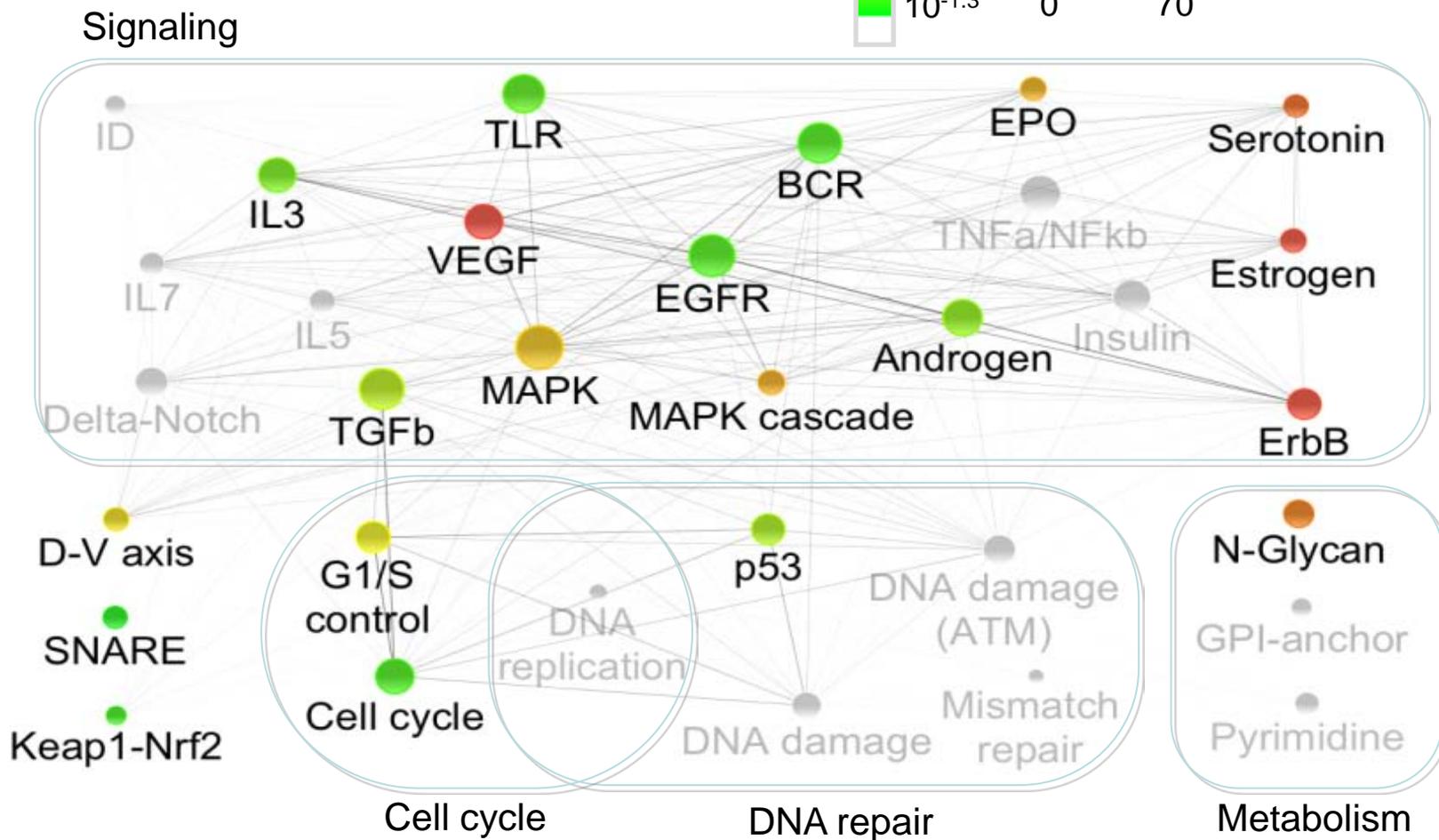
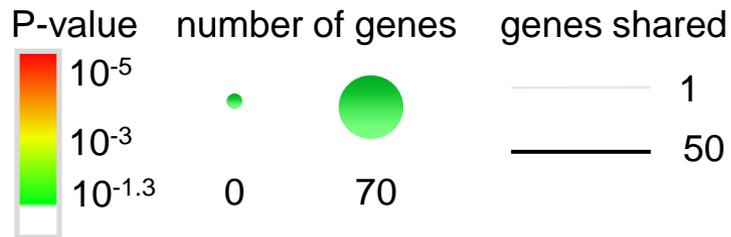
- miR-34a is located on chromosome 1p36, a candidate tumor suppressor locus deleted in neuroblastoma, breast, thyroid and cervical cancer; hypermethylated in other cancers
- miR-34a blocks cell cycle progression at the G1/S boundary
- Depending on context, miR-34 over-expression causes cell cycle arrest, senescence or apoptosis
- miR-34a is induced by p53 and is part of the p53 response (but can also be activated independently of p53)

miR-34a reduces basal activation of ERK and AKT and the proliferative response to serum growth factors



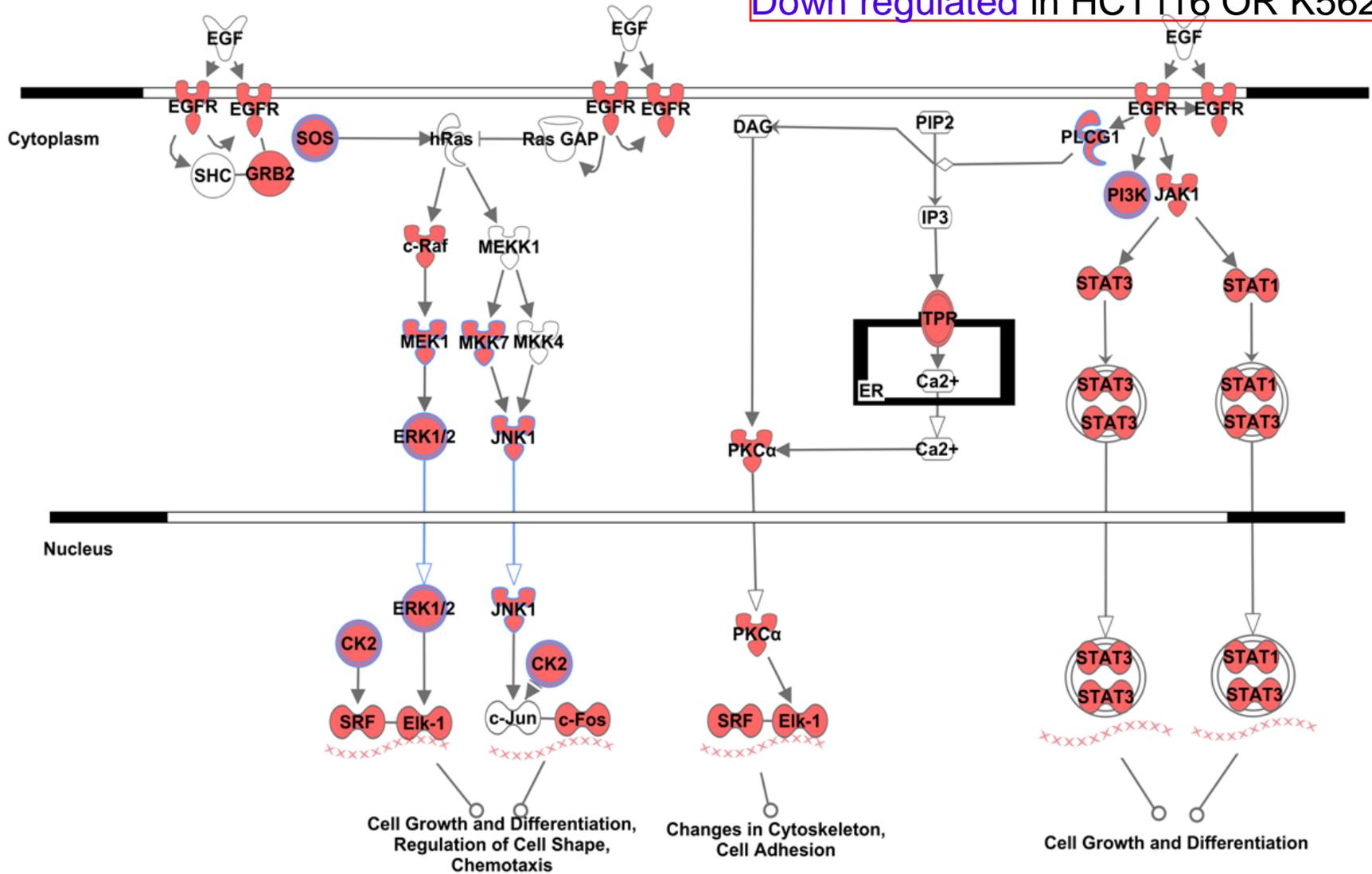
mRNAs that bind to miR-34a are enriched for growth factor signaling and cell cycle pathways

(HCT116 cells)



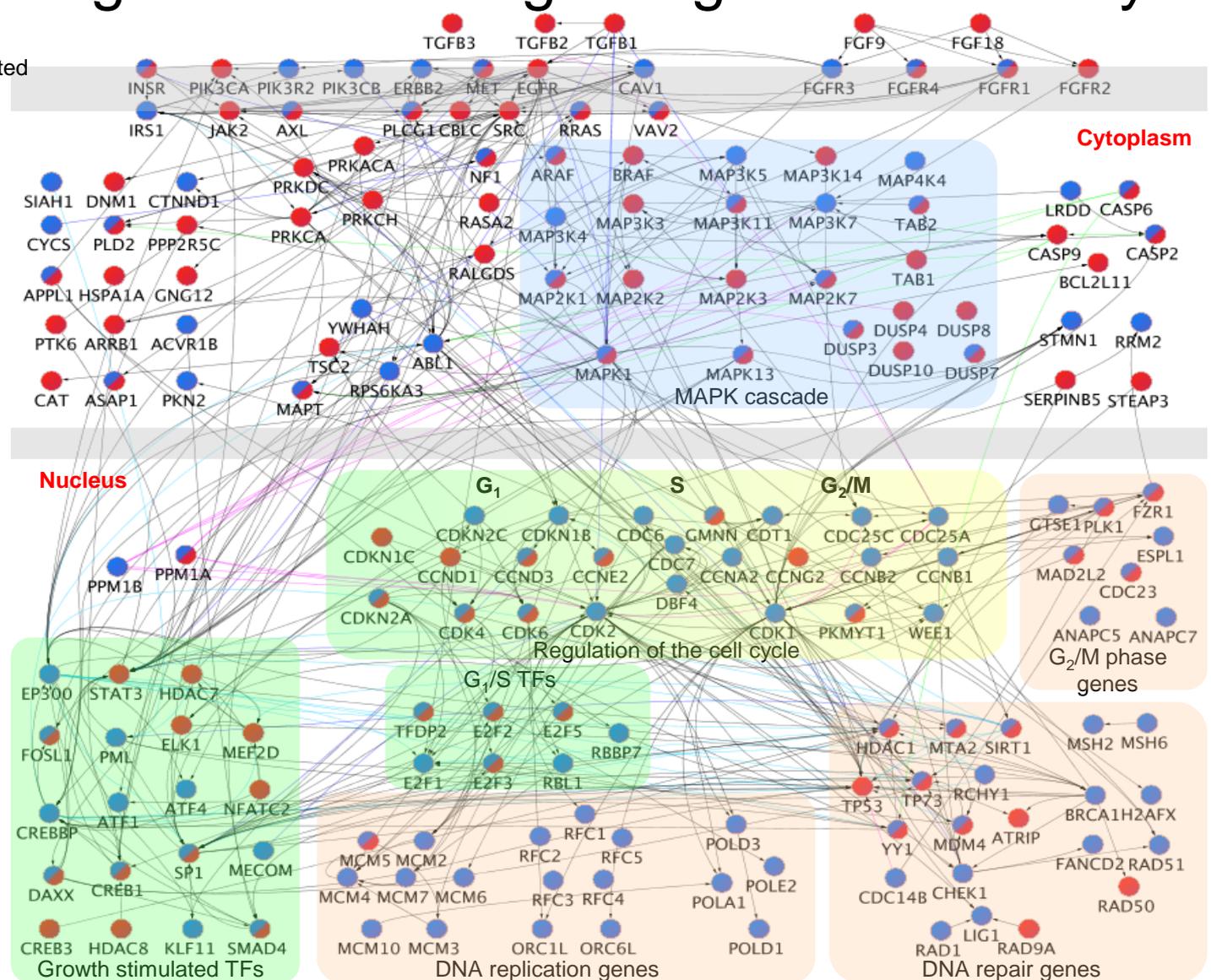
Canonical EGFR Signaling Pathway

Pulled down in HCT116 OR K562
Down regulated in HCT116 OR K562



miR-34 regulates a dense network of genes involved in growth factor signaling and the cell cycle

- Pulled-down
- Down-regulated
- Pulled-down and down-regulated



Network of genes regulated by miR-34a in 2 unrelated cancer cell lines (HCT116 and K562).
A Lal et al, PLoS Genetics 2011

Delivery.....Delivery....Delivery.....

Intracellular delivery of RNAs across the cell membrane into the cytoplasm where the RNAi machinery resides is the main therapeutic obstacle to siRNA and miRNA-based drugs.

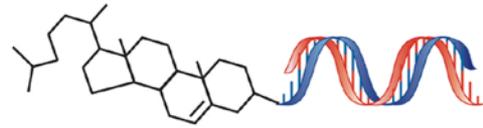
Even if the RNA is taken up by the cell, release from endosomes is a critical bottleneck.

Targeted delivery to cancer cells would be optimal – lower dose, lower toxicity to normal dividing cells.

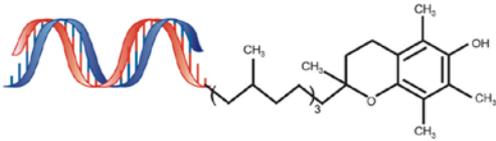
Toxicity can in principle also be reduced by targeting genes or pathways to which a specific cancer cell type is addicted.

Designs for targeted delivery

Conjugates



cholesterol

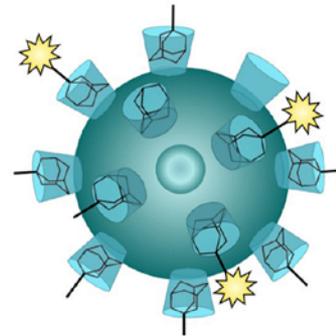
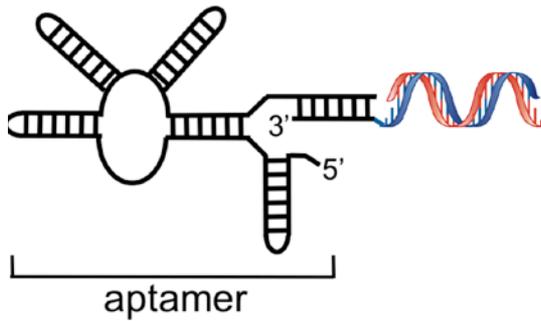


α -tocopherol



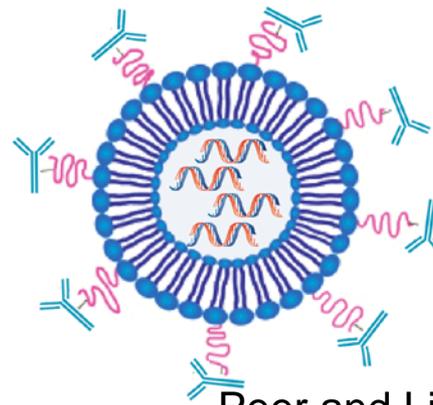
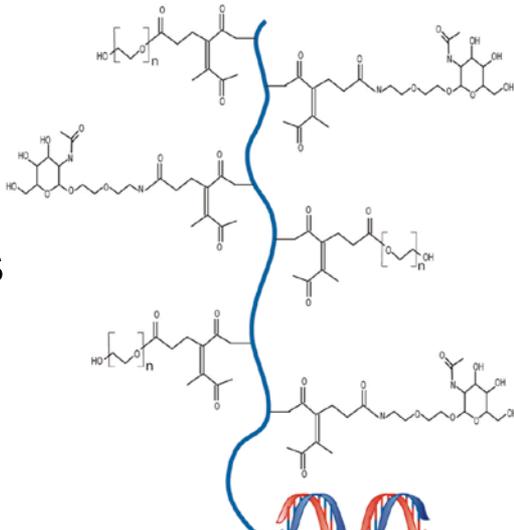
Targeting peptide-RNA binding fusion proteins

Aptamer-siRNAs



Targeted nanoparticles

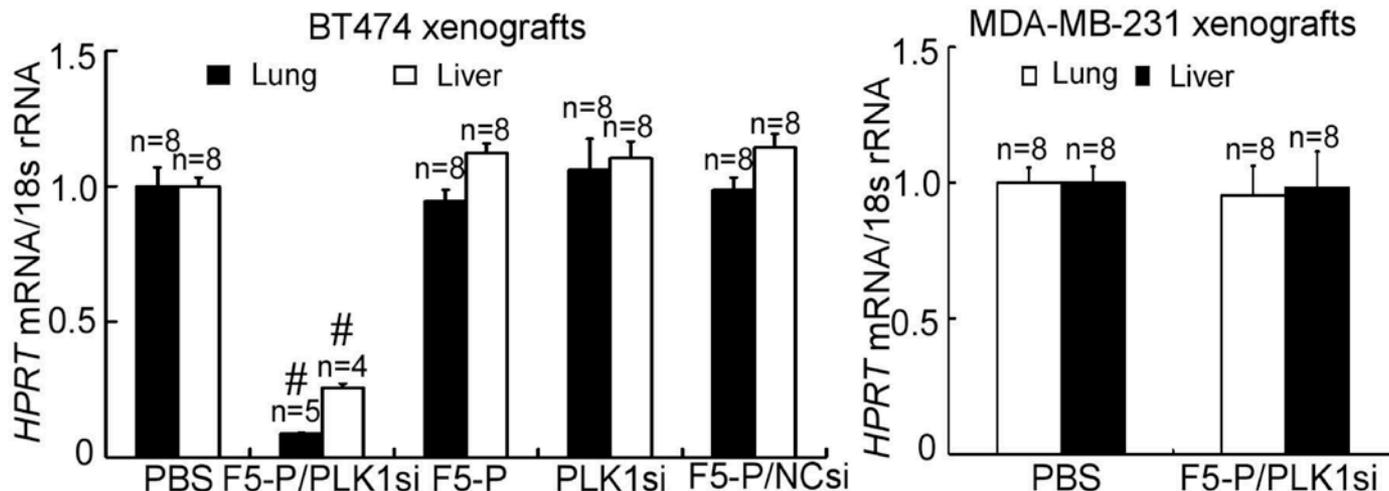
Polymers



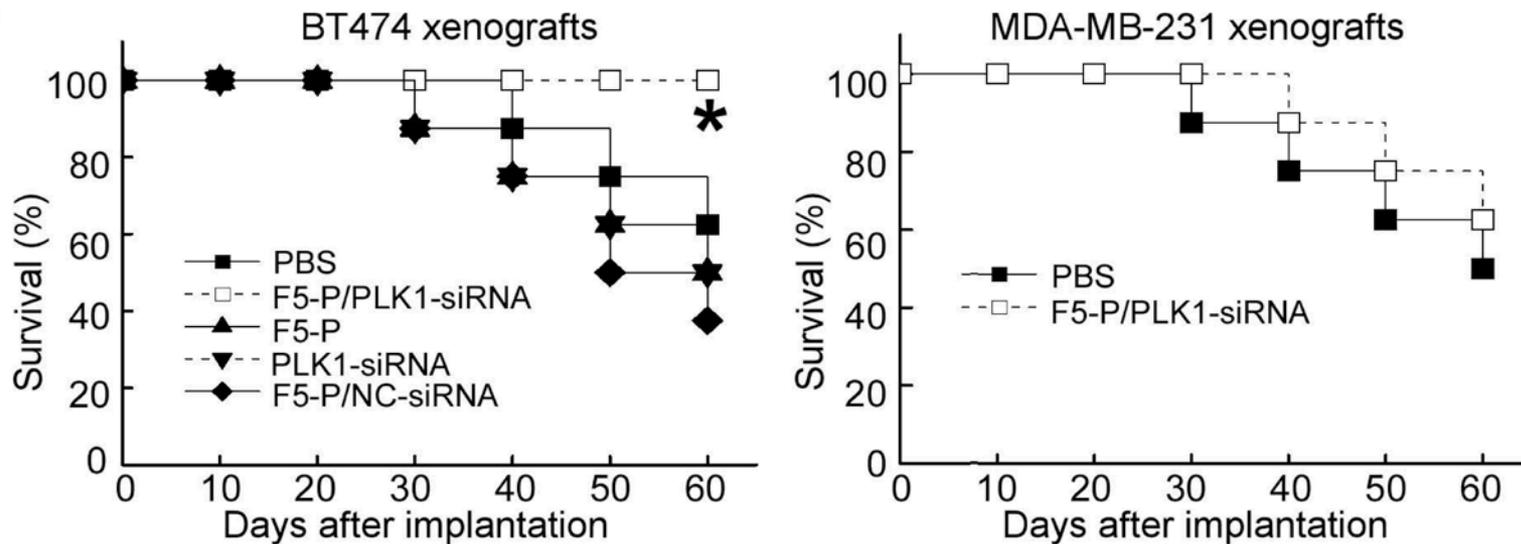
Immunoliposomes

Suppression of metastasis after iv injection of tumor cells

Reduced human HPRT gene expression in lung and liver



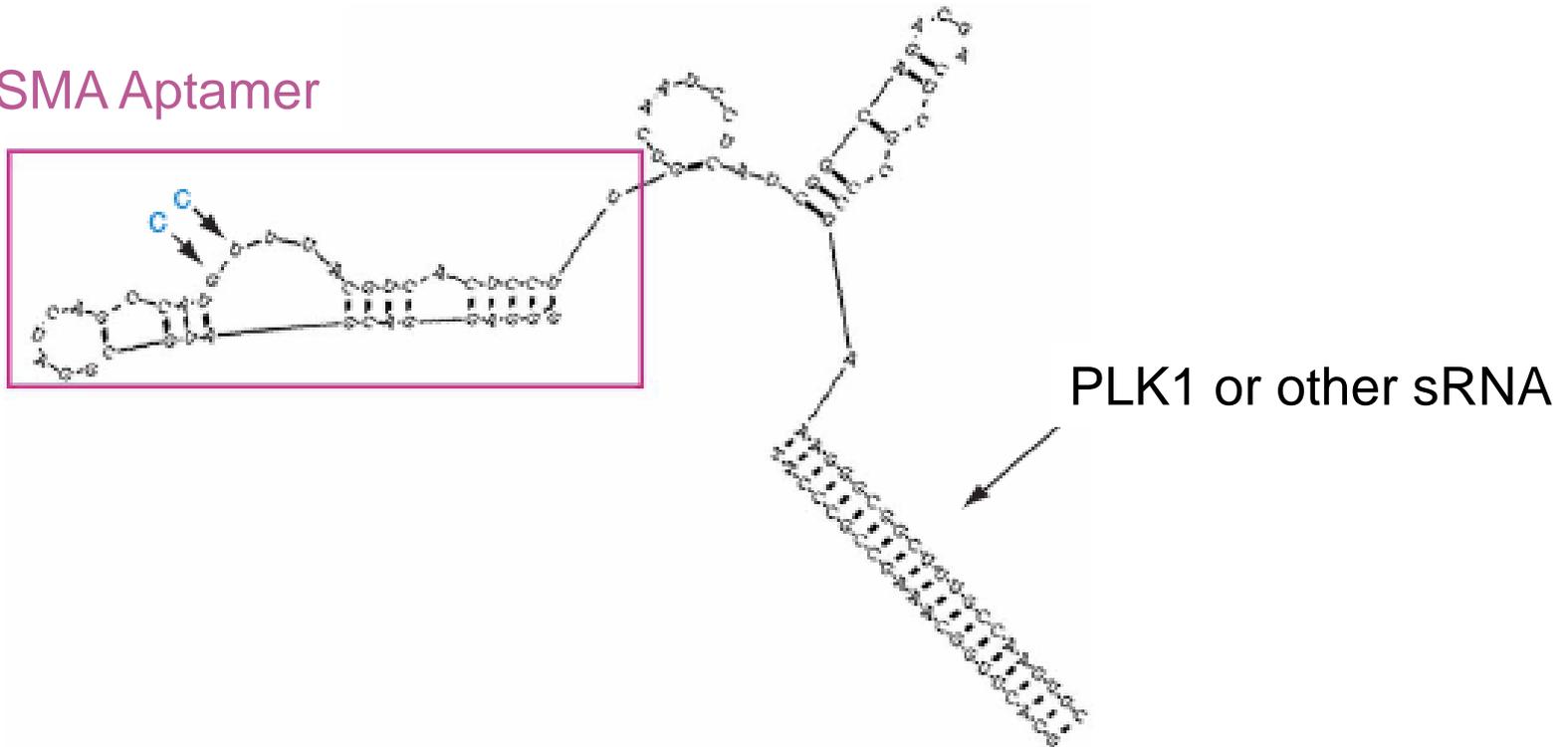
Improved survival after iv injection of Her2+ tumor



Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras

James O McNamara II^{1,3}, Eran R Andrechek^{2,3}, Yong Wang¹, Kristi D Viles¹, Rachel E Rempel², Eli Gilboa¹, Bruce A Sullenger¹ & Paloma H Giangrande¹

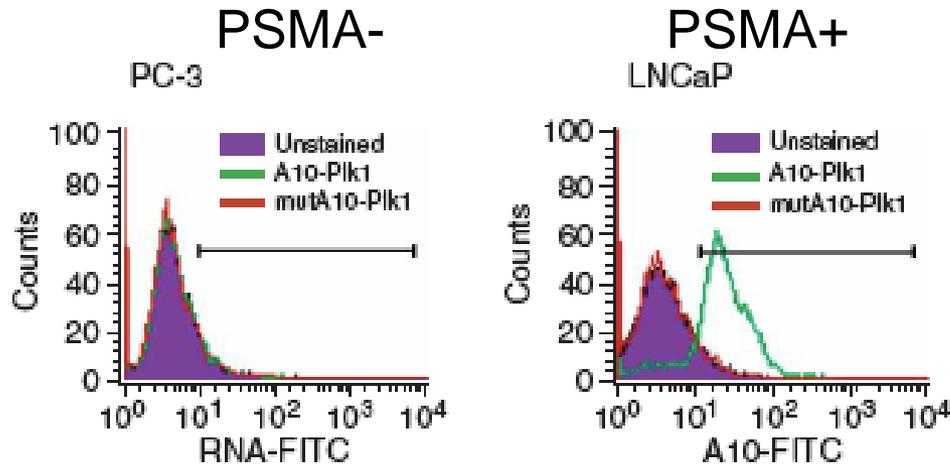
PSMA Aptamer



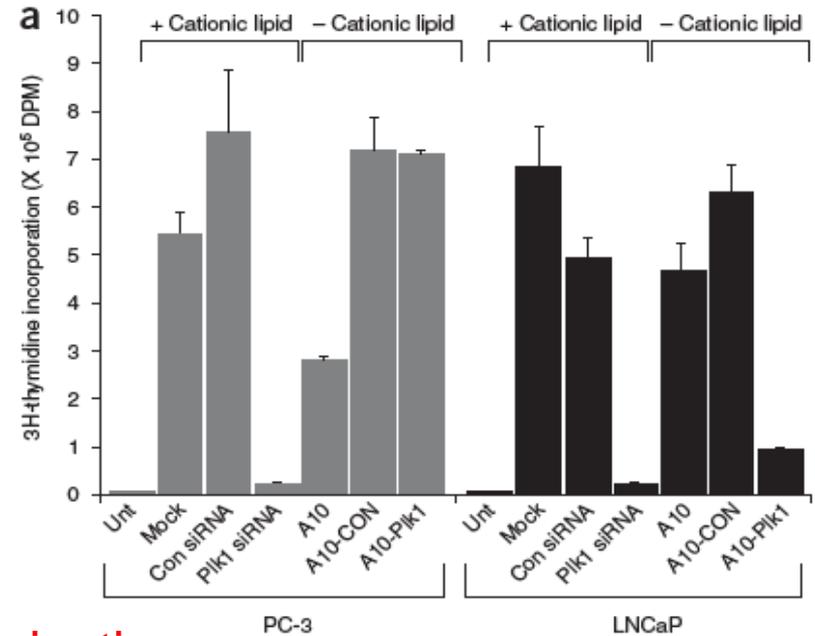
A10-Plk1 aptamer siRNA chimera binds to prostate surface membrane antigen (PSMA) and activates gene knockdown specifically on prostate cancer cells.

PSMA Aptamer-siRNA has a specific antitumor effect

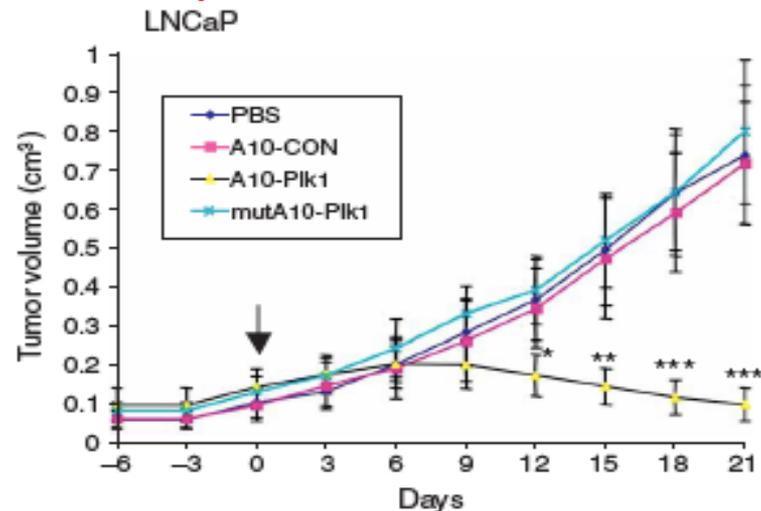
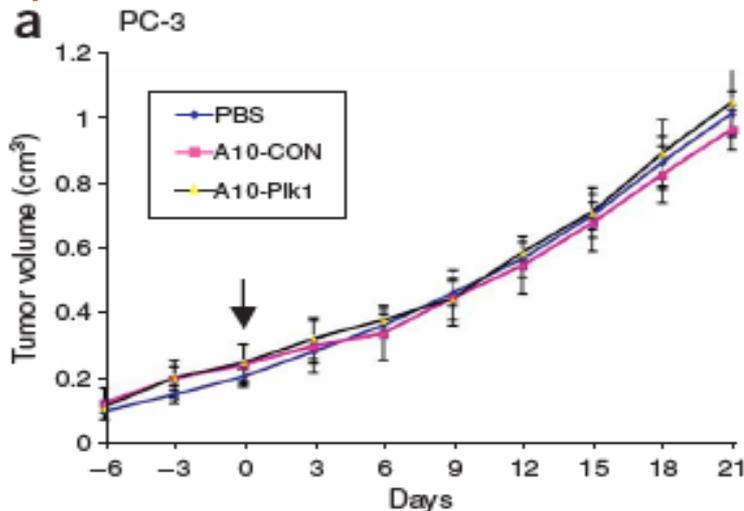
Specific binding to PSMA+ cell



Specific PLK1 Knockdown

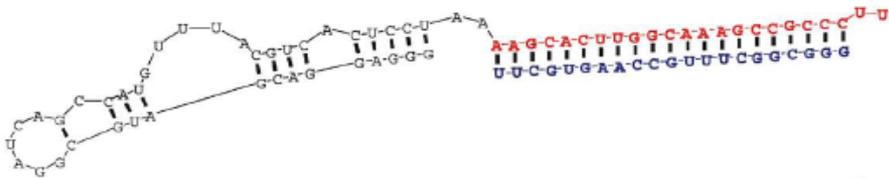


Significant tumor effect after intratumoral injection



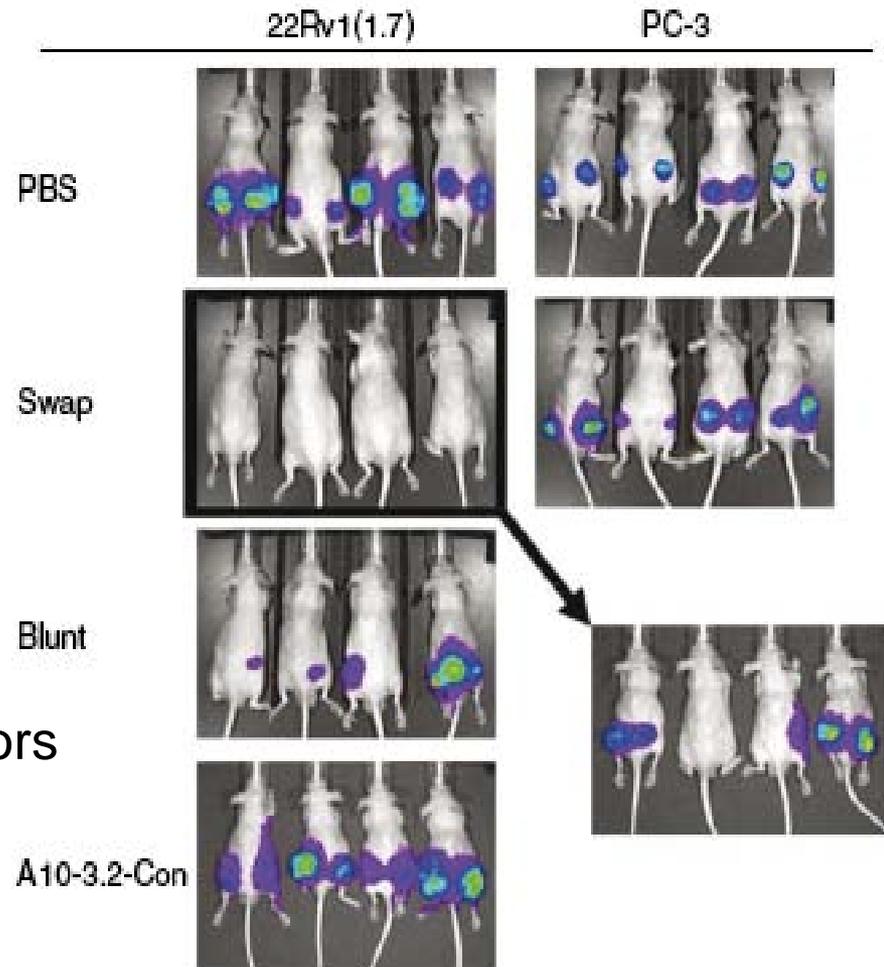
Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors

Justin P Dassi^{1,2,5}, Xiu-ying Liu^{1,5}, Gregory S Thomas^{1,2,5}, Ryan M Whitaker¹, Kristina W Thiel¹, Katie R Stockdale¹, David K Meyerholz³, Anton P McCaffrey^{1,2}, James O McNamara II¹ & Paloma H Giangrande^{1,2,4}



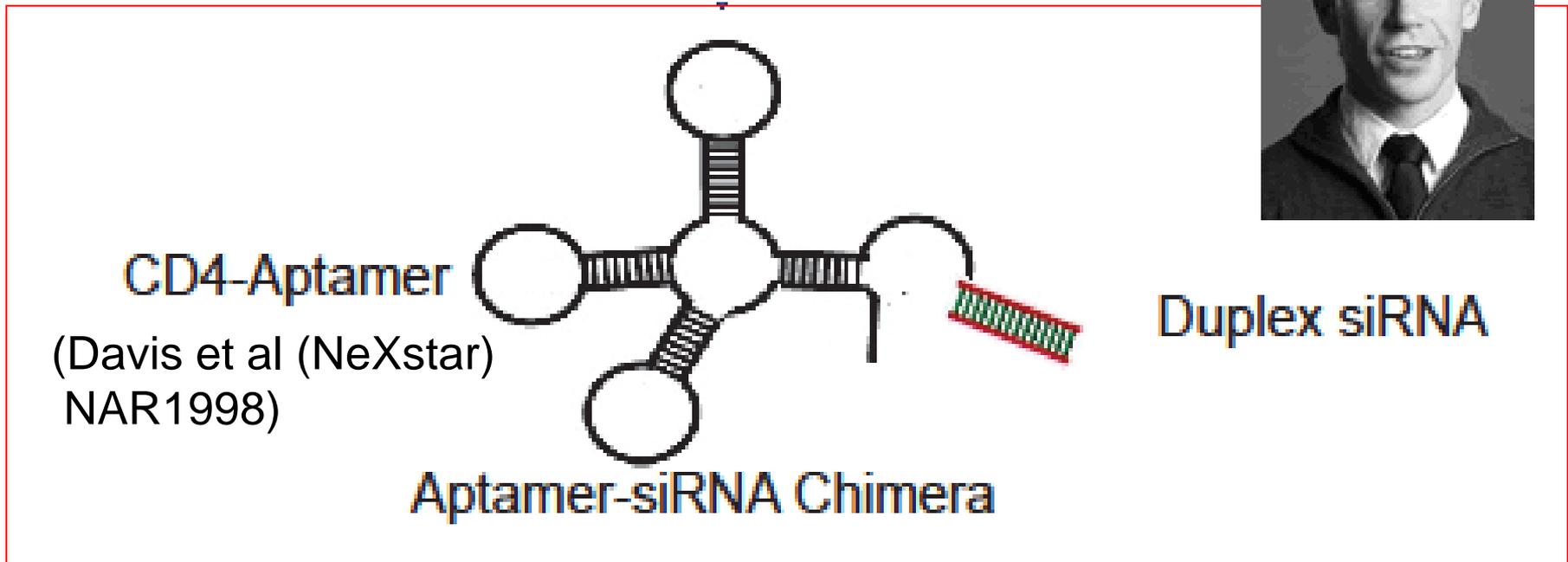
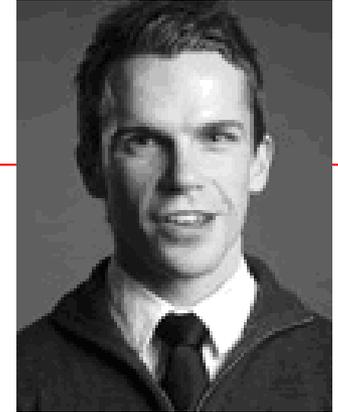
- optimized aptamer (subnanoM affinity)
- optimized siRNA
- added PEG for better circulating half-life

Specific antitumor effect in PSMA+ tumors
(1 nmol qd iv x 10)

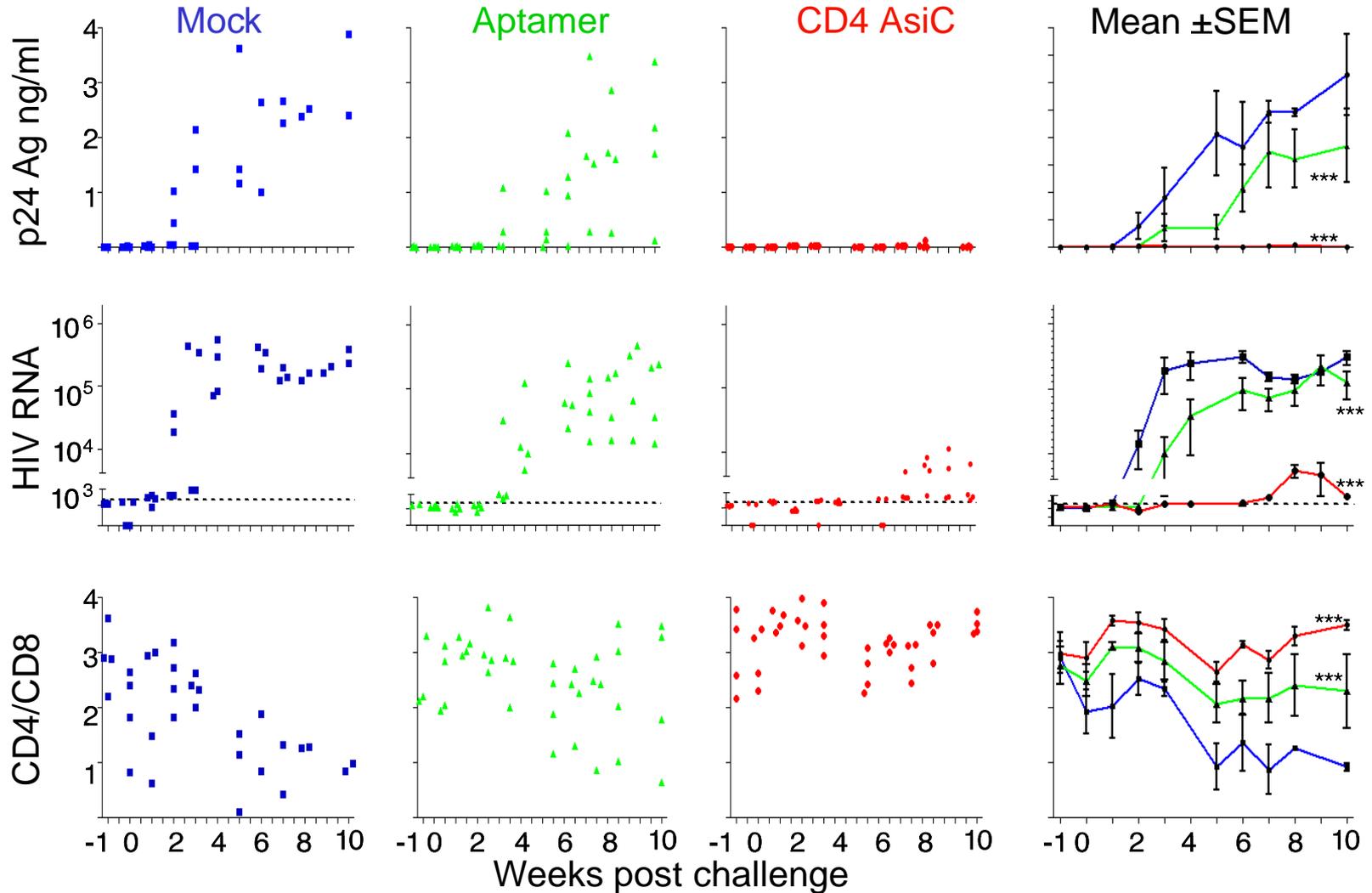
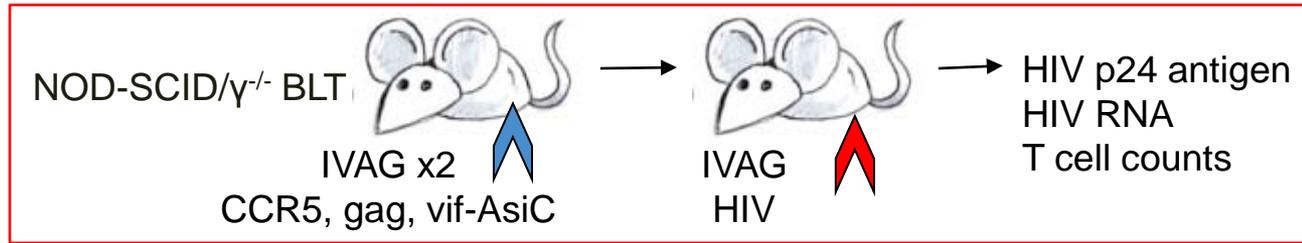


RNA aptamers deliver siRNAs to the immune cells that HIV infects

Lee Wheeler



In vivo protection from HIV sexual transmission



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