

Nanoparticle Targeted siRNA Cocktail Therapeutic Translation:

The Next “Antibody”

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Chief Executive and Scientific Officer

The logo for AparnaBio features a stylized, calligraphic letter 'A' in red and blue, followed by the word 'AparnaBio' in a blue serif font.

AparnaBio Overview

➤ History

- Founded mid-2007 to develop biomedical nanoparticles, in Maryland
 - M. Woodle and P. Scaria
 - Serial entrepreneurs with track record in nanoparticles & nucleic acids
 - Management team with biotech & pharma experience
- Growing IP portfolio: issued patents and applications
- Government, academic and industry collaborations and funding

➤ Technology and Product Pipeline

- AparnaBio NanoElectroPlex™ platform, *InVivoPlex*™ reagents
- Tissue Selective Nucleic Acid Nanoparticles
 - *InVivoPlex*™ reagent family for *in vivo* gene function research
 - Versatile oriented antibody targeting
 - Tissue targeted therapeutics (anti-angiogenesis) and vaccines
- Antifungal therapeutic for invasive infections
 - Based on histatin-like activity of nanoparticle carrier

RNAi: Rapid Target Validation ⇒ Therapeutics

Nature Review, Drug Discovery, Oct. 2005

INFECTIOUS DISEASE

Faster drugs for unknown bugs



Emerging infectious diseases pose the threat of a looming global pandemic. As illustrated by the outbreak of the SARS coronavirus (SCV) in 2003, there is a pressing need for new effective treatments that can be rapidly developed in response to previously unknown viruses. Reporting in *Nature Medicine*, Li *et al.* took a step towards this goal by demonstrating that intranasal delivery of SARS-specific small interfering RNA (siRNA) was effective against SARS in a rhesus macaque model — providing hope that siRNA could provide the means to move rapidly from gene sequence to targeted therapeutics for many previously intractable diseases.

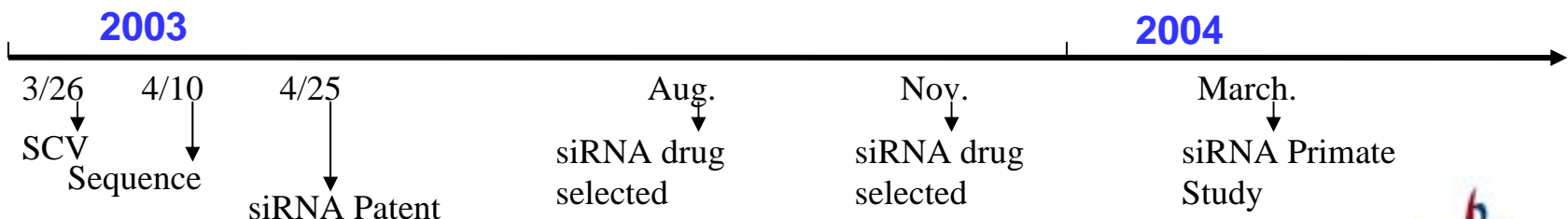
The recently established rhesus macaque SARS model, based on intranasal instillation of a highly virulent SARS strain, mirrors the sequence of pathologies in humans with SARS. Taking advantage of previously identified siRNA sequences that have a synergistic anti-SCV effect *in vitro*, the authors used a clinically approved aqueous carrier solution (DSW) to intranasally administer the therapeutic siRNA either 4 hours before, concurrently or 4 hours after infection of the animals with SCV. The effectiveness of this treatment was evident as

macaques from all three treatment regimes had a lower increase in body temperature, reduced viral loads and a reduced level of lung damage, indicating potent suppression of SCV-induced SARS pathology compared with SCV-infected animals that received no siRNA or unrelated siRNAs.

These results might be attributable to a protective effect conferred by siRNA on cells against SCV infection (the prophylactic treatment could even lead to direct degradation of viral RNA by pre-existing siRNA in the upper airway tract upon entry of the viral particle); the inhibition of viral protein synthesis in infected cells caused by the degradation of SCV mRNA; or preventing the replication of the SCV genome and the spread of virions to uninfected cells. As neutralizing antibodies were also detected soon after infection, the authors speculated that several antiviral mechanisms were operative.

Importantly, the effect of the siRNA did not seem to be caused by the induction of a pro-inflammatory interferon response, which can potentially exacerbate symptoms and lung damage. Specific emphasis was placed on tolerability and safety — as

A Speedy Process:



siRNA Drugs? – Key “Platform” Properties

- ❖ **Biologic Origin of Function (Endogenous Mechanism)**
- ❖ **Versatile Yet Selective (Inhibit Nearly Any Gene)**
- ❖ **Multiple Formats, Companies (Accessible Technology)**

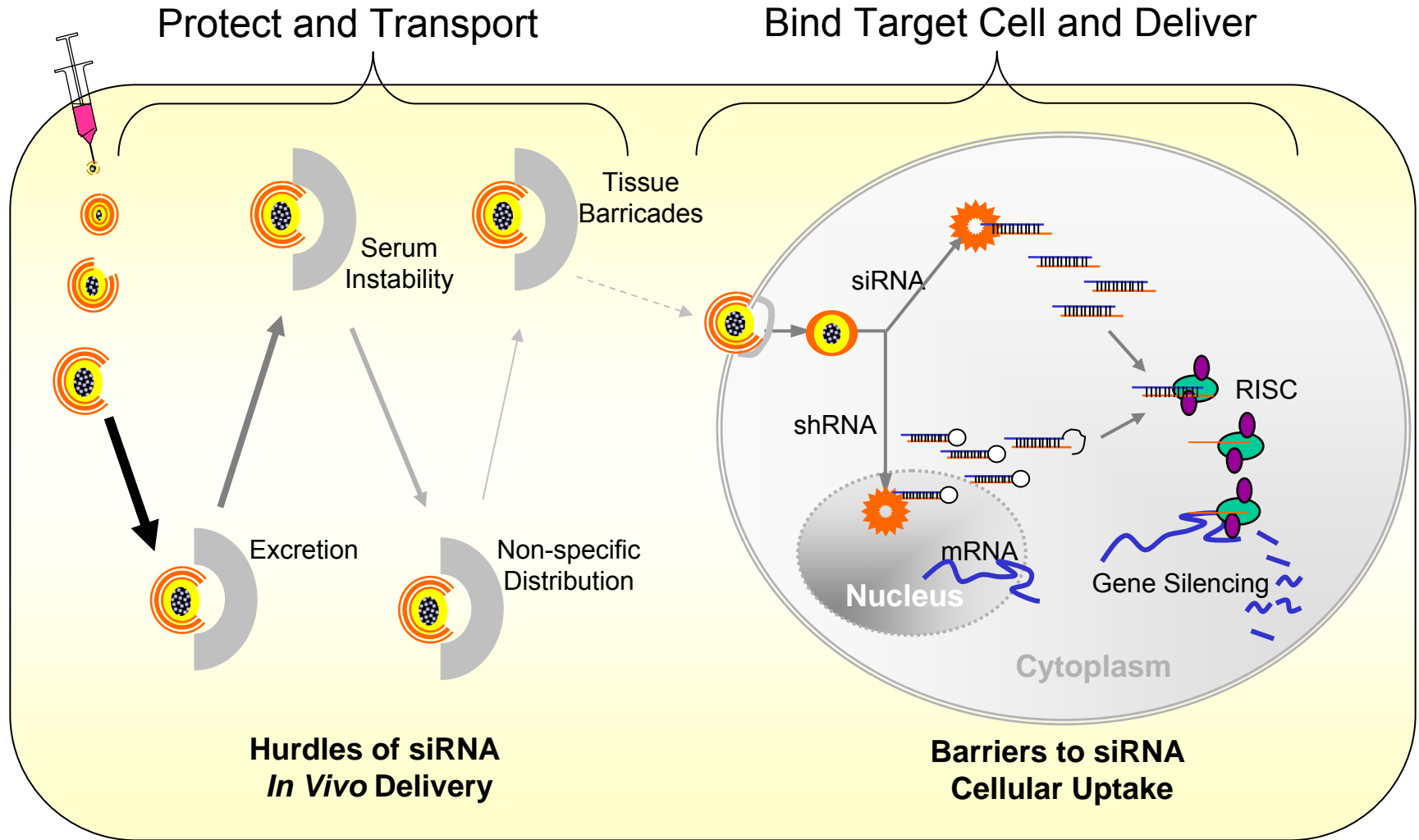
Advantages over antibodies?

- ⇒ **Inhibits expression of proteins inside the cell**
- ⇒ **Assemble multi-targeting cocktails**

But?

- ⇒ **Oligonucleotides – Delivery, Delivery, Delivery**

RNAi Nanoparticle: Bind, Transport & Deliver

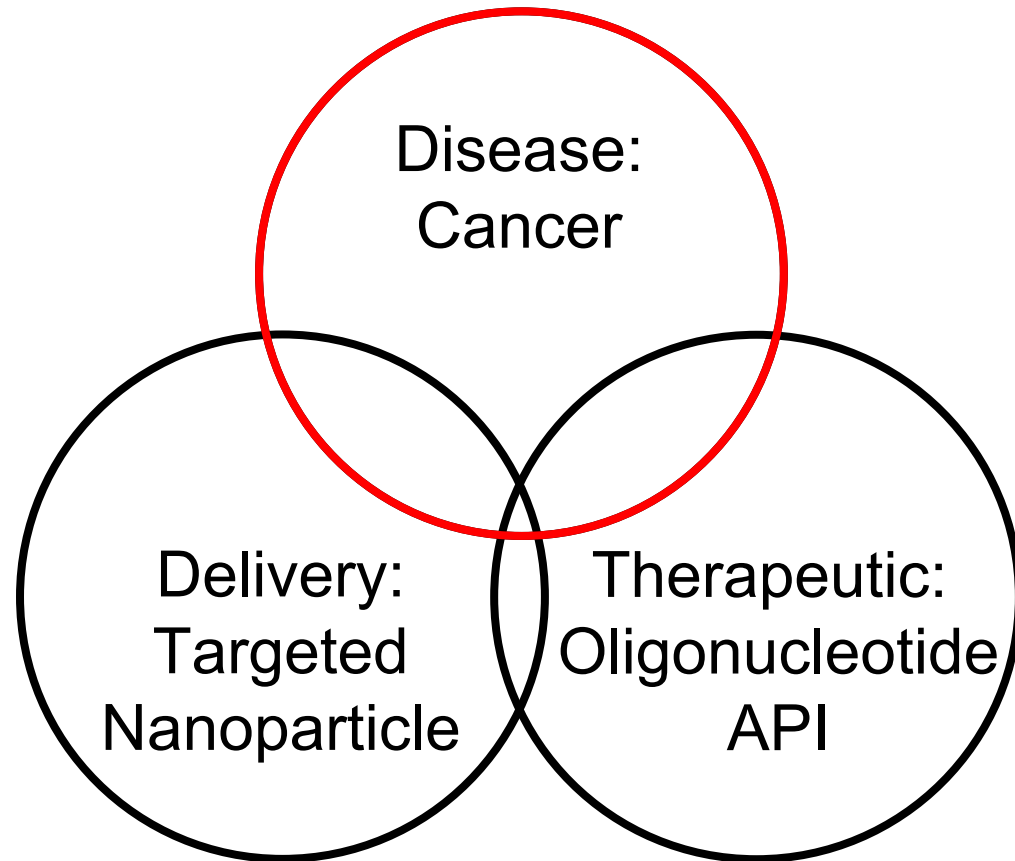


Challenges of *In Vivo* siRNA Delivery

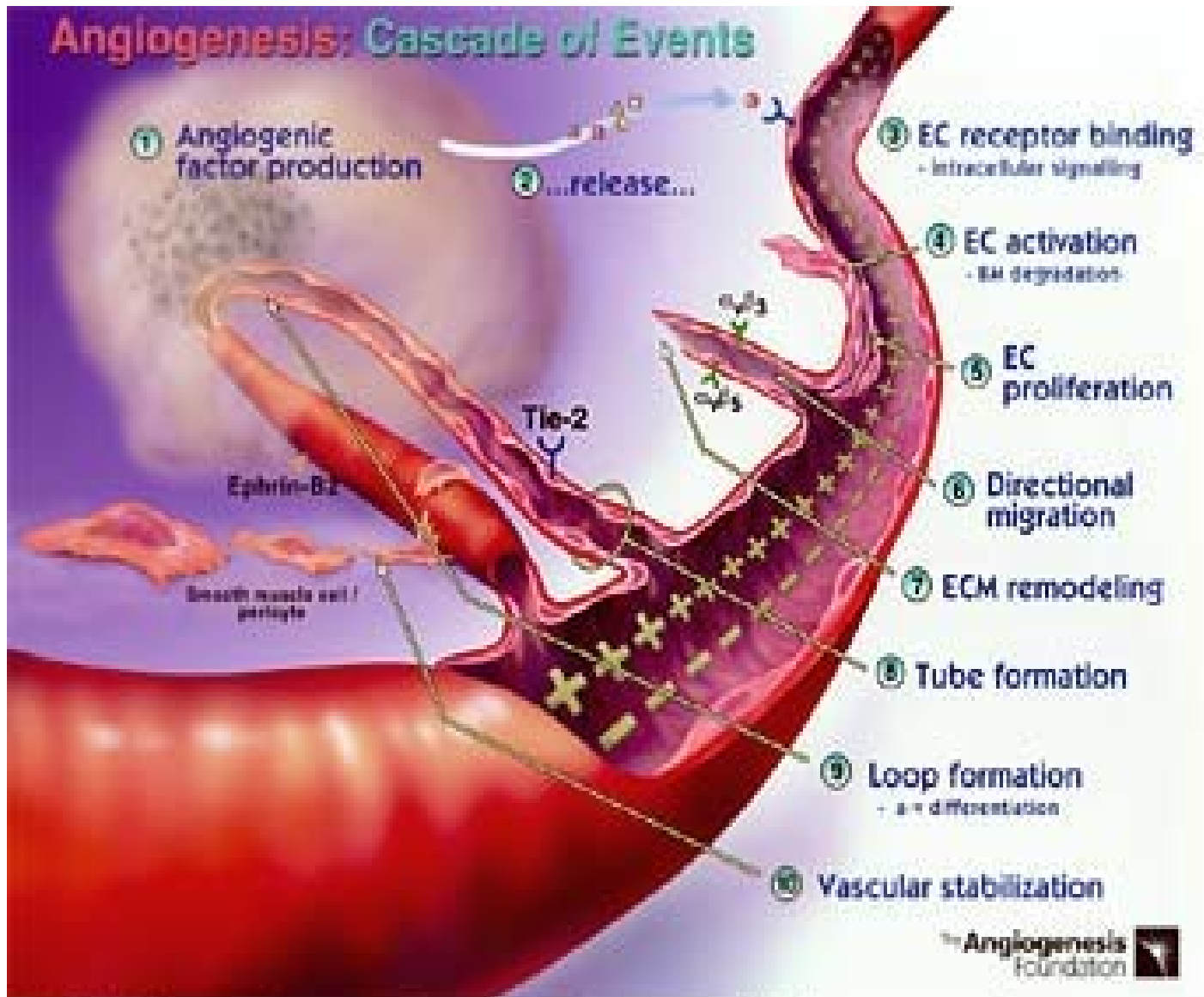
Case Study: To Pre-IND

- ❖ **Indication:** Solid tumors: renal, colon, lung, ...
- ❖ **Mechanism of Action:** Anti-angiogenesis at neovasculature
- ❖ **Active Drug Ingredient:** siRNA cocktail, VEGF pathway
- ❖ **Carrier:** cRGD peptide-polymer conjugate
- ❖ **Drug Formulation:** Nanoparticle suspension
- ❖ **Administration:** Intravenous infusion

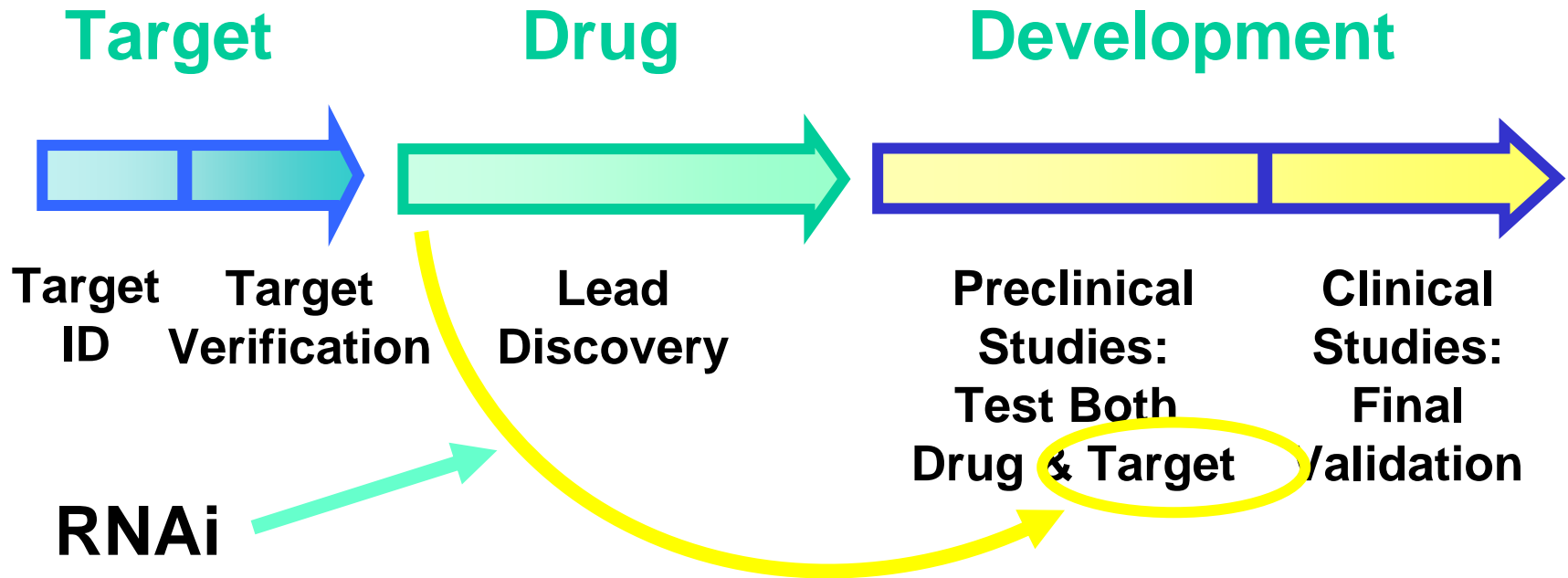
RNAi Therapy: Tumor Targeted Nanoparticles



Broad Intervention: Tumor Angiogenesis



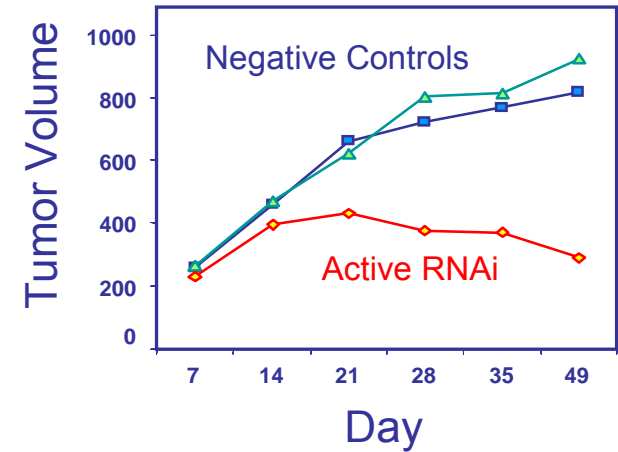
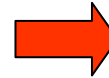
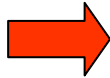
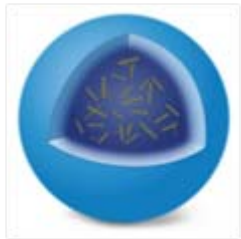
In Vivo siRNA Target Validation



Preclinical Disease Model Target Verification:

- 1) Rapidly Eliminate False Positive Targets**
- 2) Validate Combinations – MultiTarget, Target-Drug**
- 3) Validate RNAi Therapeutic Agents For Efficacy**

AparnaBio's *In Vivo* Target Research



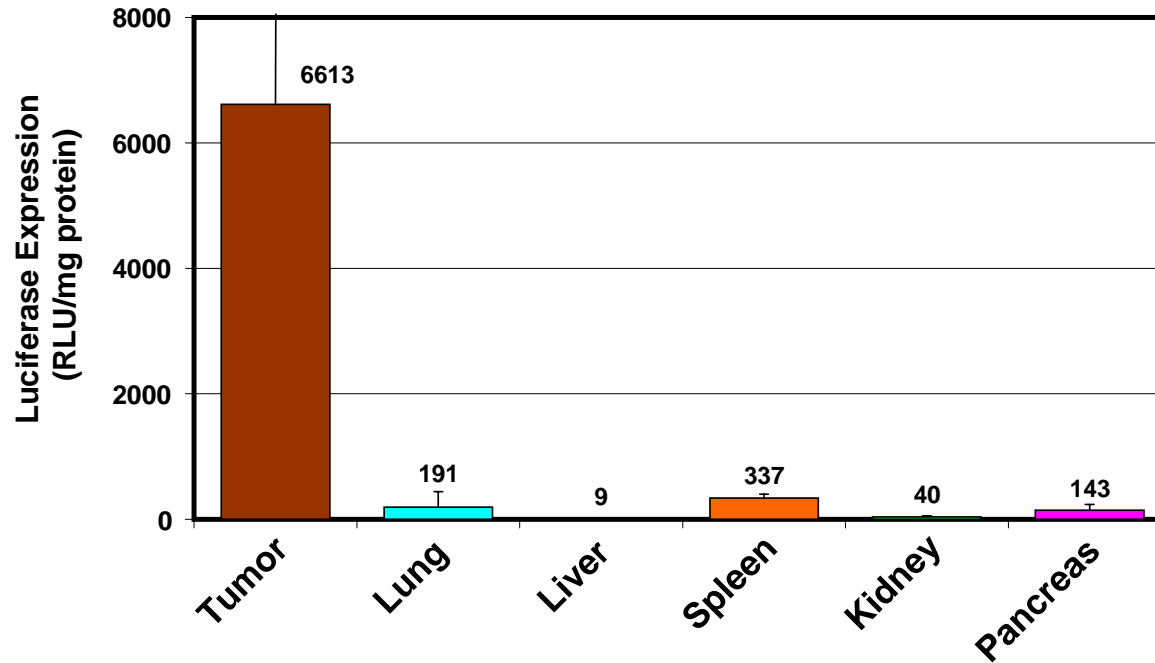
InVivoPlex®
with In Vitro
Active RNAi

Murine
Tumor
Model

In Vivo
RNAi Gene
Inhibition Effect

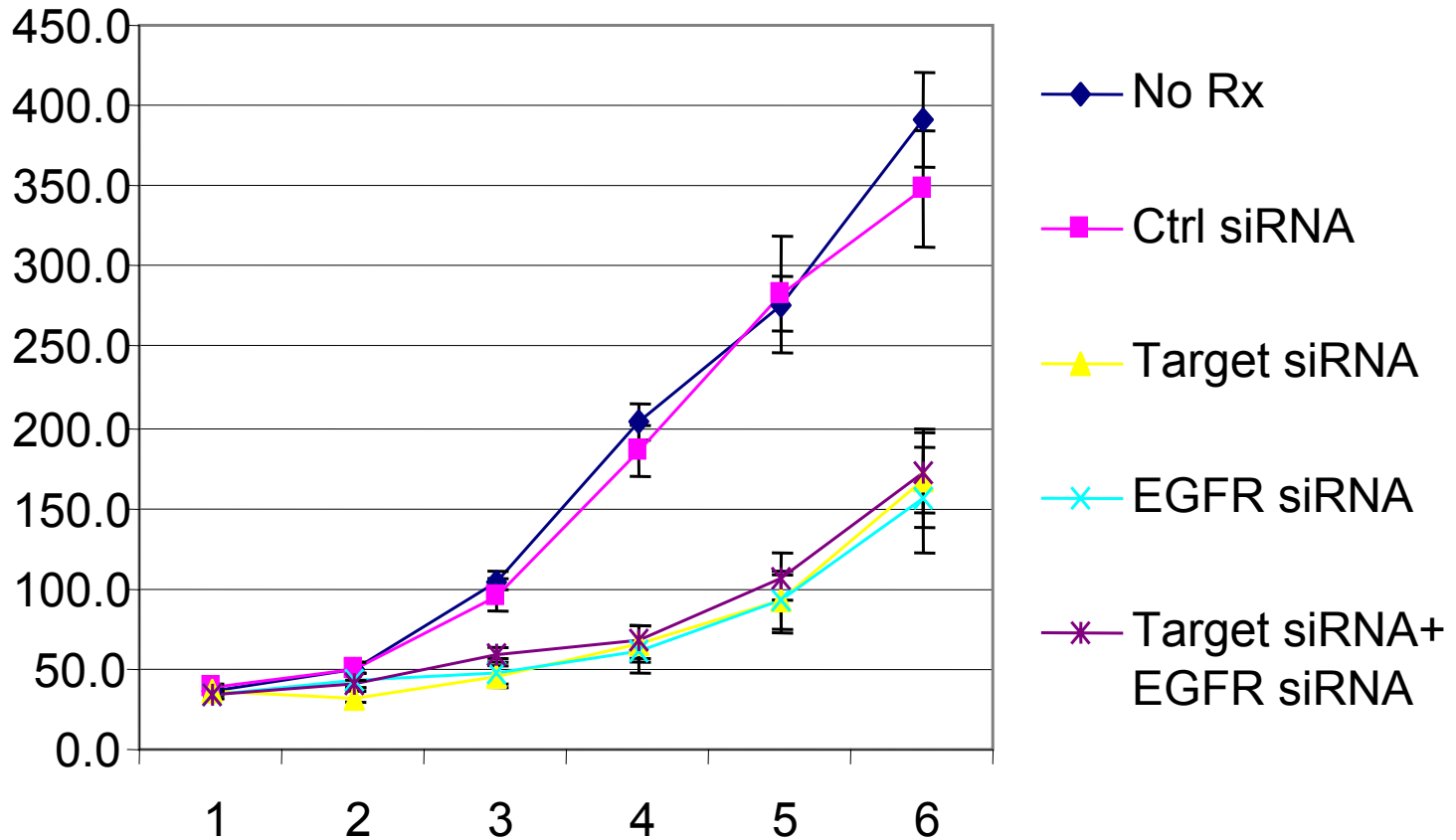
InVivoPlex[®]–TUMOR Tissue Selectivity/Activity

InVivoPlex[®] Nanoparticle Tumor Localized Activity IV administration to U87 tumor bearing mice

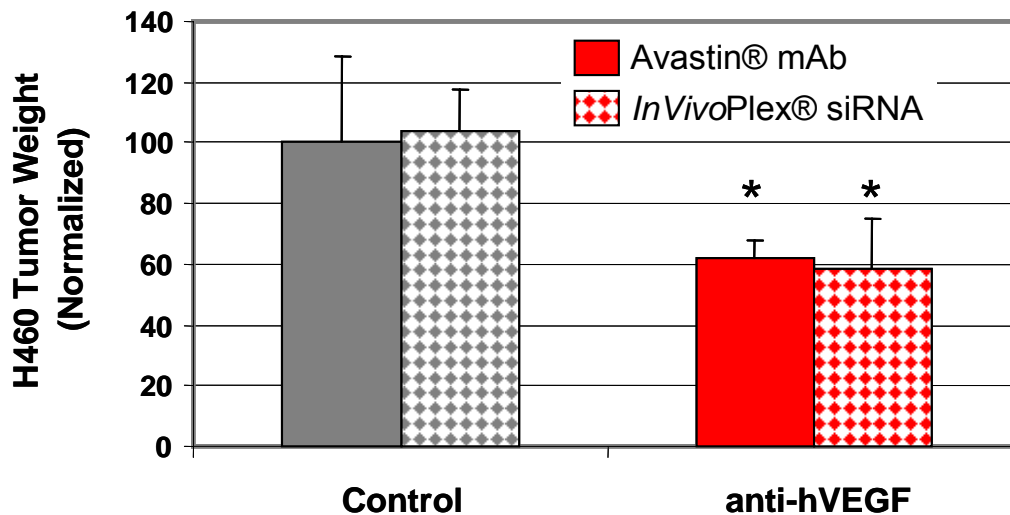
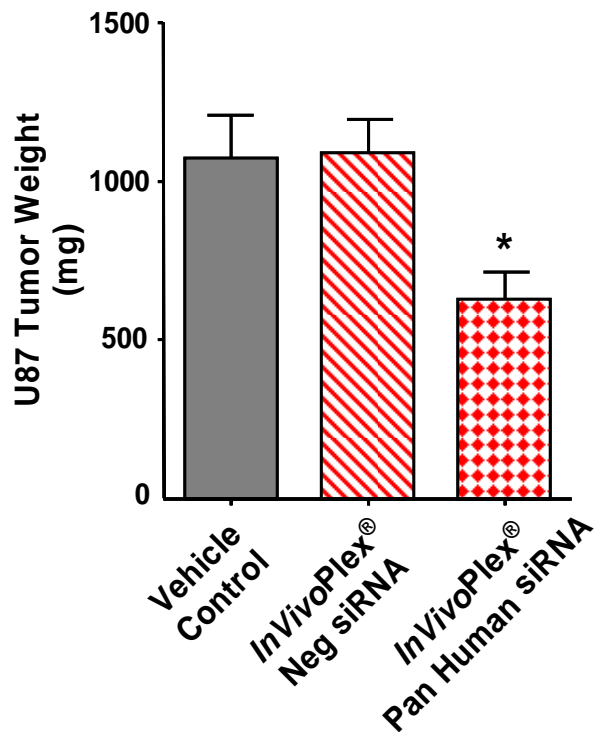


New Target vs EGFR siRNA Function

HNSCC 1483 Xenograft tumor (IV Administration)

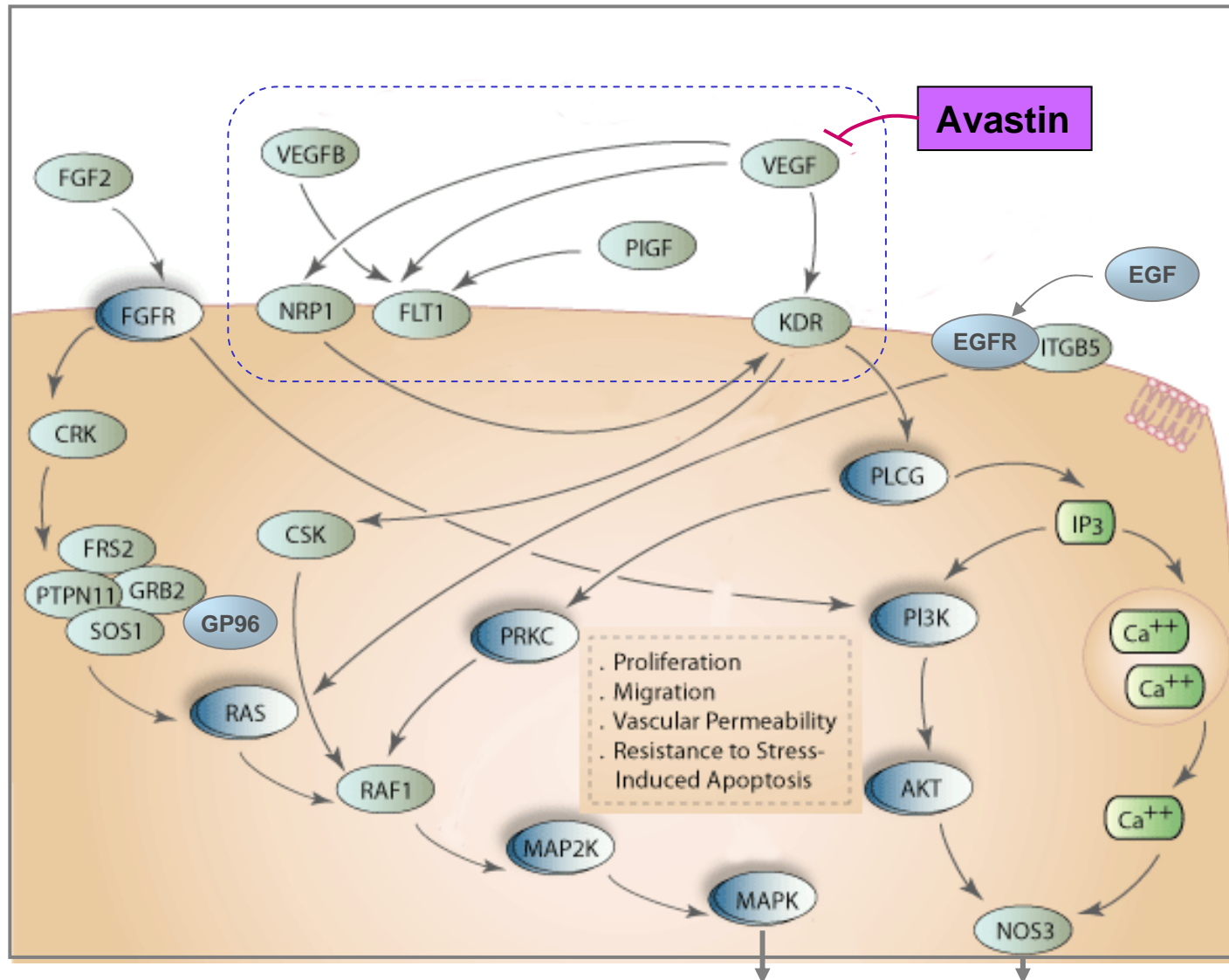


In vivo siRNA Tumor Target “Validation” by *InVivoPlex*®



InVivoPlex® siRNA nanoparticle treatment by iv, 3 times per week for two weeks, inhibits xenograft tumor growth. *Left*: inhibition of U87 subcutaneous xenograft tumors with pan human siRNA. *Right*: inhibition of H460 subcutaneous xenograft tumors with *InVivoAct*™ hVEGF – equivalent to inhibition by the approved hVEGF mAb therapeutic.

VEGF – Validated Pathway/Target



Cancer: Multi-Targeting Needed

“Fresh approaches are needed for antiangiogenesis therapies ... ”

“Knocking down multiple regulators of angiogenesis ...”

Masters of angiogenesis

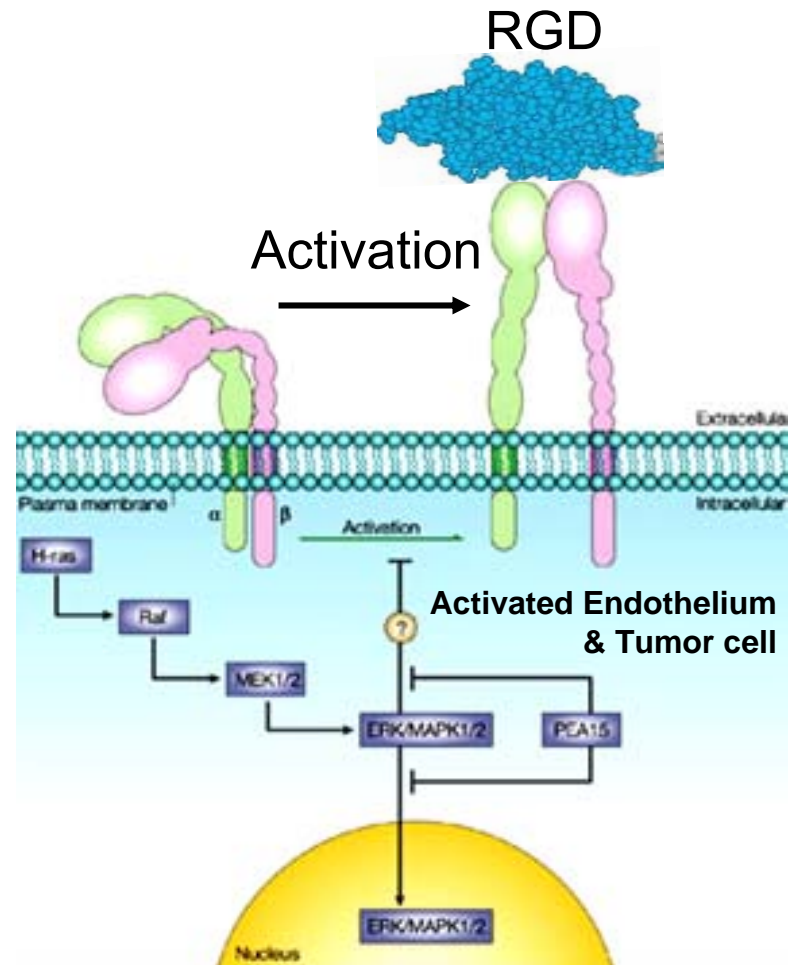
Robert M Strieter,

Departments of Medicine, Pathology and Laboratory Medicine, and
Pediatrics, Division of Pulmonary and Critical Medicine, David Geffen
School of Medicine at UCLA

Nature Med. (2005) 11, 925-927.

$\alpha v \beta 3$ Integrin: Tumor Neovasculation

- ❖ The involvement of integrins in vascular proliferation, adhesion, and wound repair has been well documented.
- ❖ The integrin receptor, $\alpha v \beta 3$, selectively expressed on growing blood vessels and tumor cells, has been one of the most intensely studied of the integrin receptors.
- ❖ A well-defined ligand to $\alpha v \beta 3$ integrin is the Arg-Gly-Asp (RGD) peptide (Cheresh, 1991).



Clinically Validated Strategy

❖ Anti-Angiogenesis:

- Recent Success in Cancer and Ocular Therapeutics
 - Avastin, Macugen, etc.

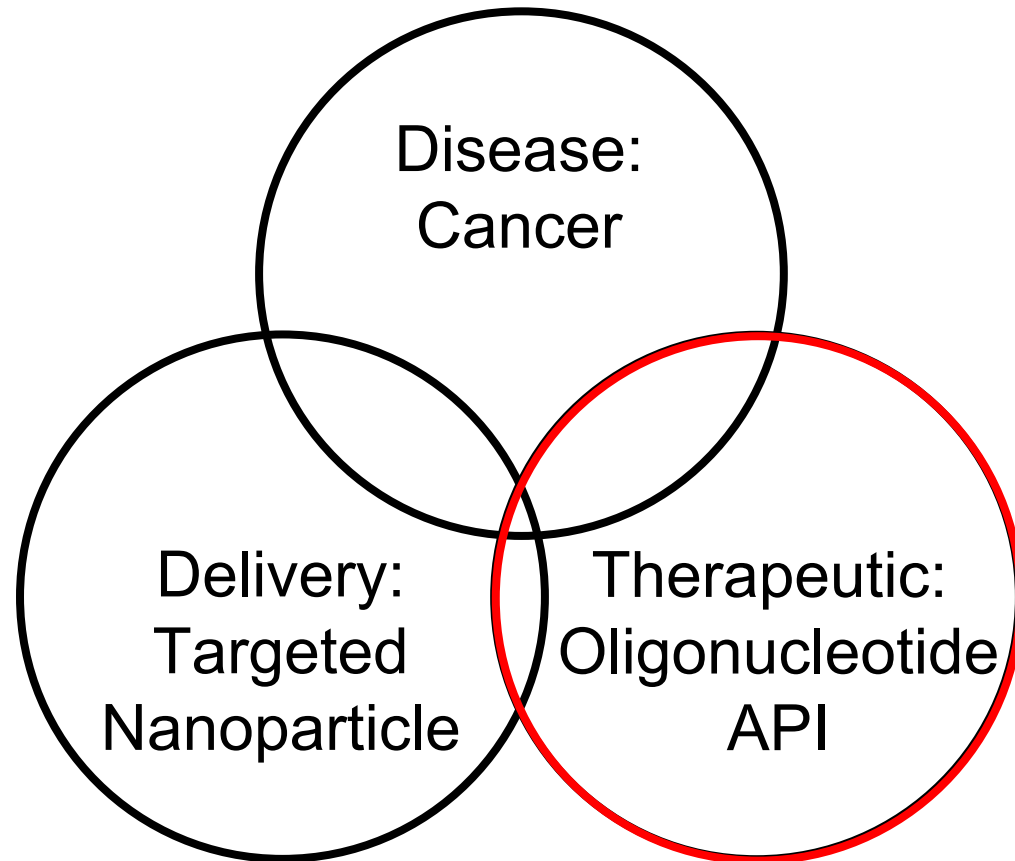
❖ VEGF Pathway Targeting:

- VEGF Inhibitors Proven For Clinical Benefit (VEGF mAb & aptamer)
- VEGF Pathway Inhibitors
 - PTK787, CEP-7055, SU-11248 receptor antagonists

❖ Tumor Neovasculature Targeting via Integrin Ligand:

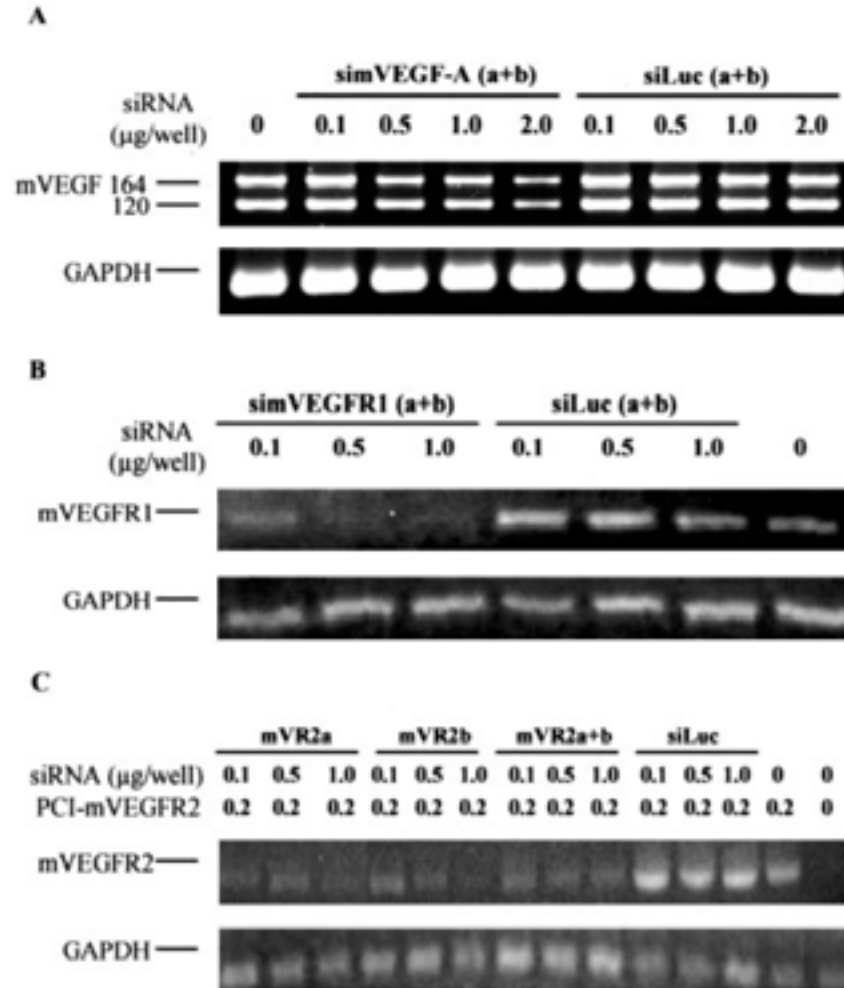
- $\alpha v\beta 3/\alpha v\beta 5$ Integrin Specificity for Tumor Validated Clinically:
 - Cilengitide (RGD peptide) and Vitaxin (mAb) clinical studies
- “2-C” Form of RGD Peptide Validated $\alpha v\beta 3$ Selective Binding

RNAi Therapy: Tumor Targeted Nanoparticles

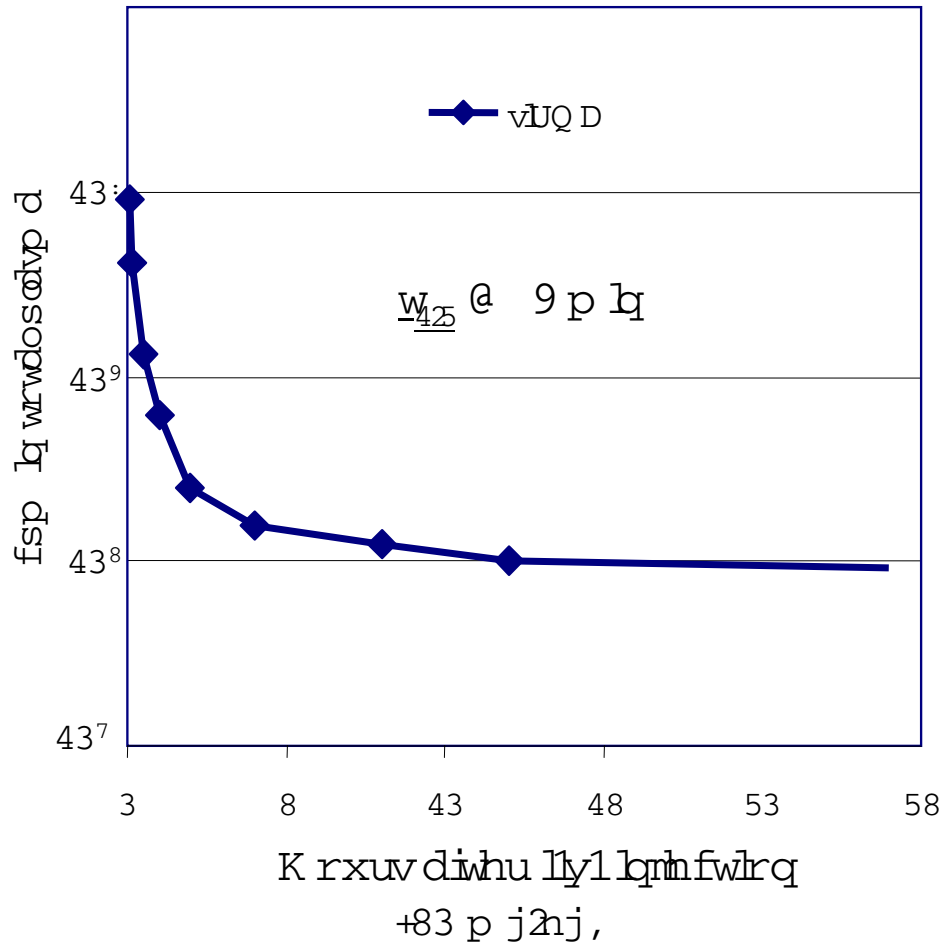


Potent siRNA API

In Vitro

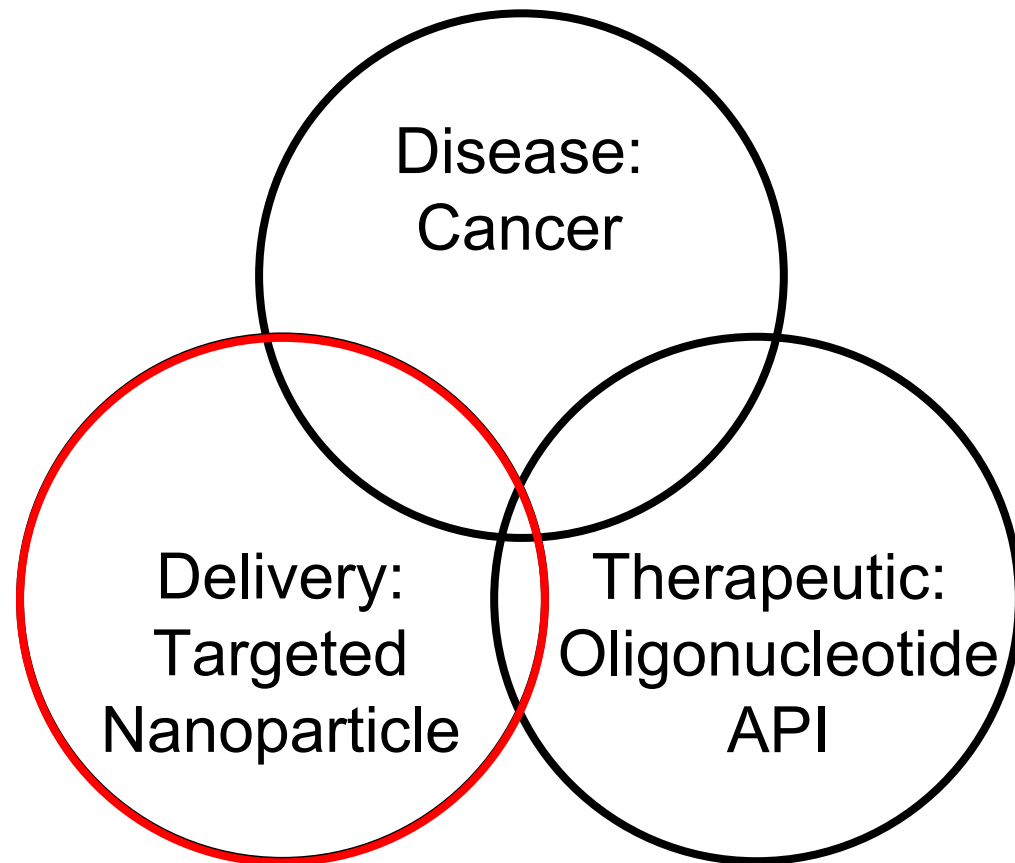


siRNA – Rapid (Urinary) Clearance, Mostly Intact



Adapted From Alnylam's Presentation

RNAi Therapy: Tumor Targeted Nanoparticles



Nanomedicines in Clinical Use

❖ Liposomes

- e.g. Doxil, DaunoXome, Myocet, Ambisome

❖ Polymeric Nanoparticles

- e.g. Abraxane

❖ Polymer Conjugates

- e.g. Oncaspar, Neulasta, PEG-intron, Adagen, Copaxone

❖ Antibody Conjugates

- Drug Conjugates, e.g. Mylotarg, ...
- Radioisotope Imaging, e.g. Zevalin, Tositumab, Bexxar

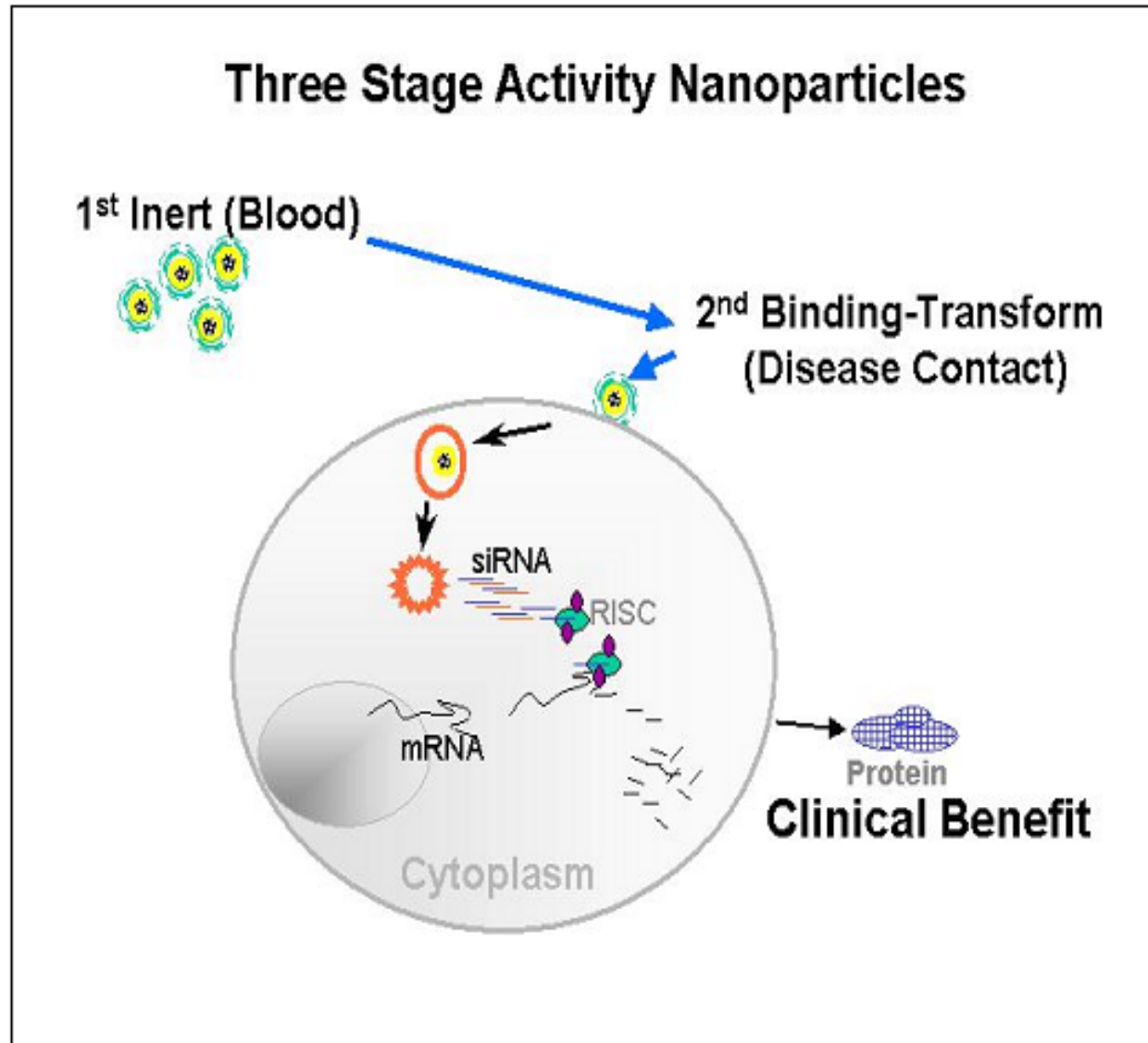
Nanoparticle Therapeutics: Successful Approach

Liposomal Chemotherapy – Delivery Success

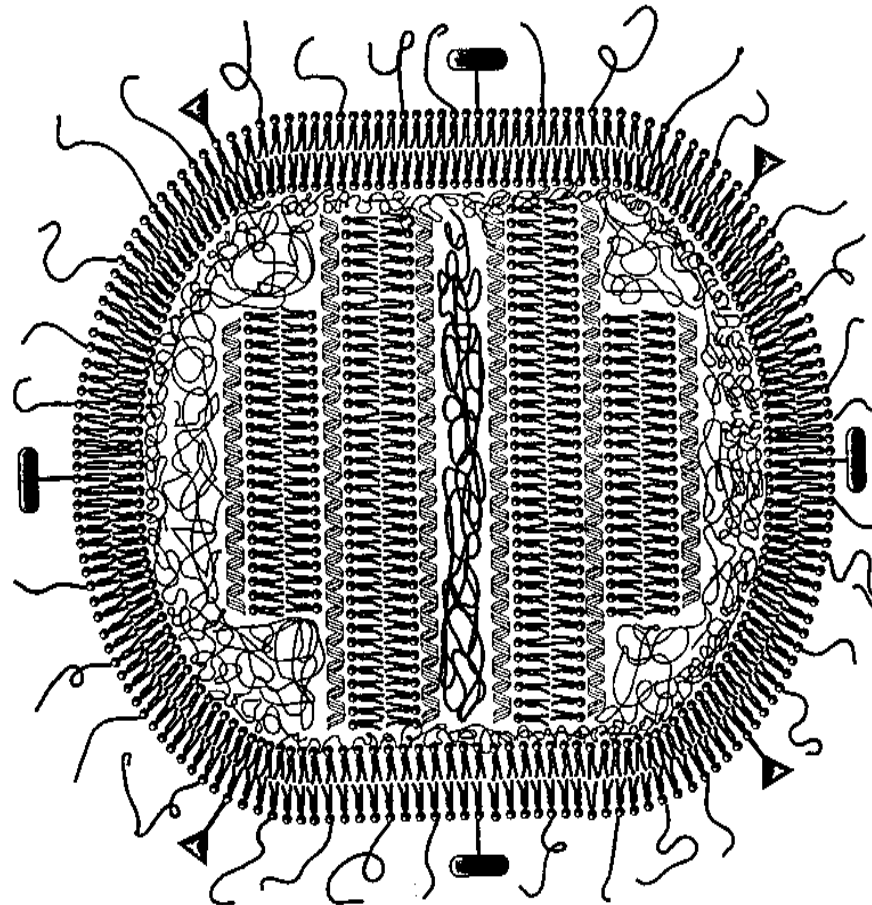


Multiple Indications, Large Markets

Apply Lessons To Deliver Nucleic Acids?

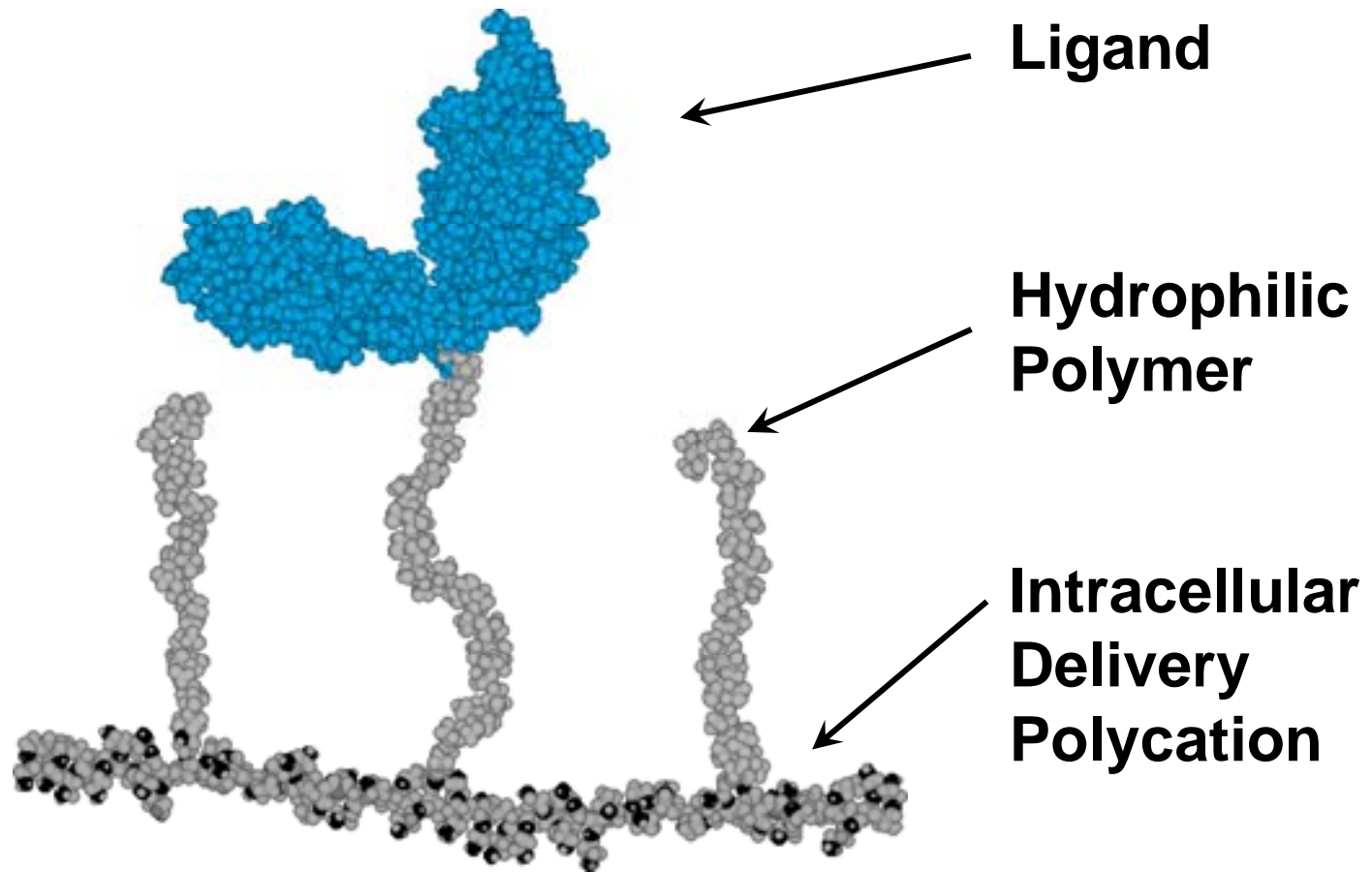


Nucleic Acid Agents: Synthetic Virus Concept

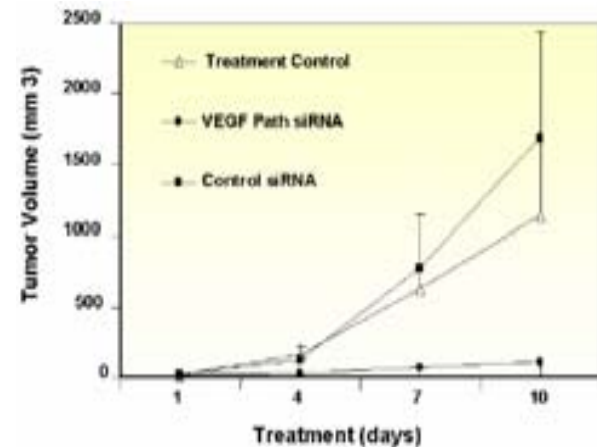
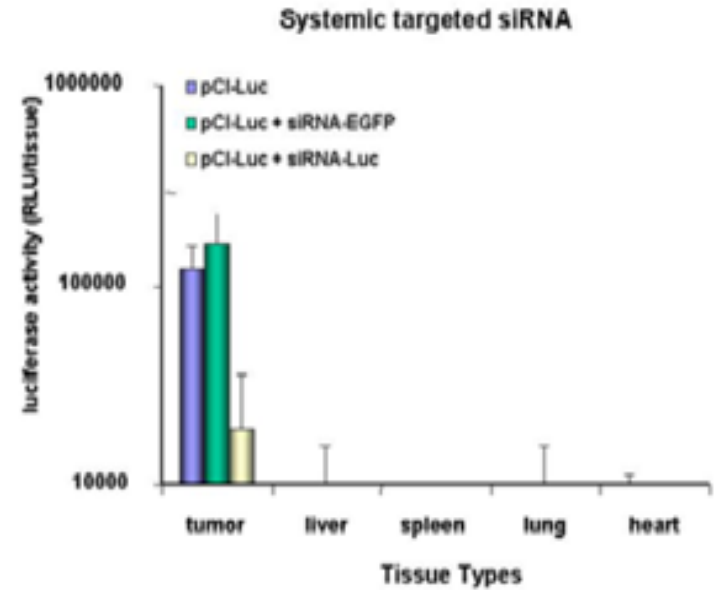
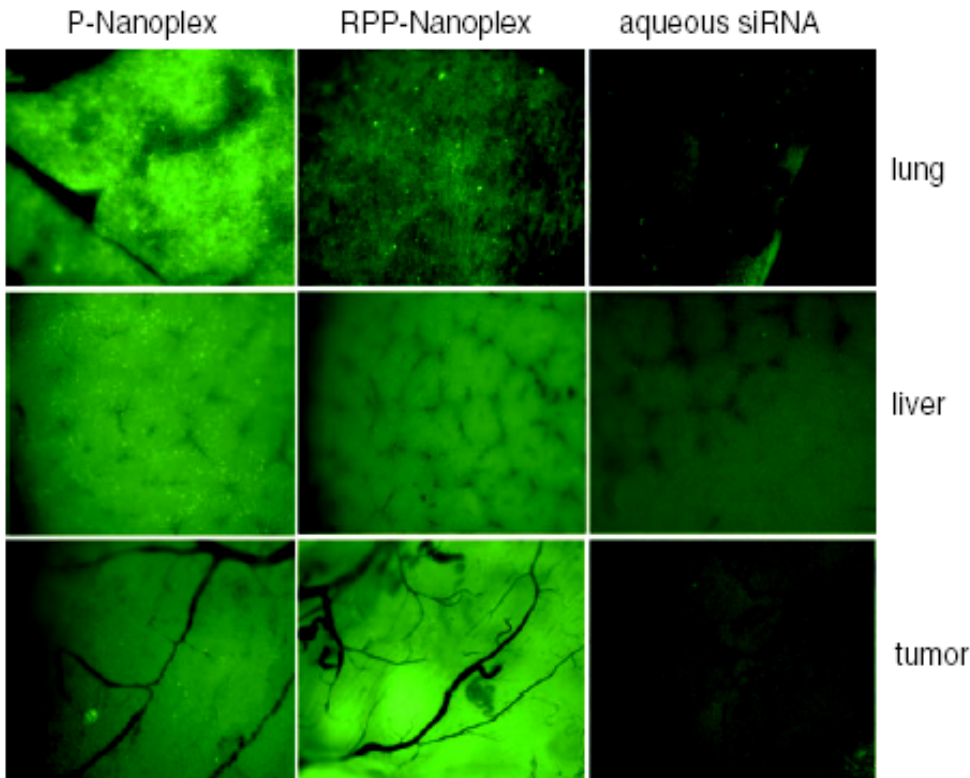


From Lasic, 1997, Liposomes in Gene Delivery, CRC Press

Nanoparticle Modular Chemistry



cRGD Angiogenesis Targeted siRNA Delivery



(*Nucleic Acids Research*, 2004, Vol. 32, No. 19).

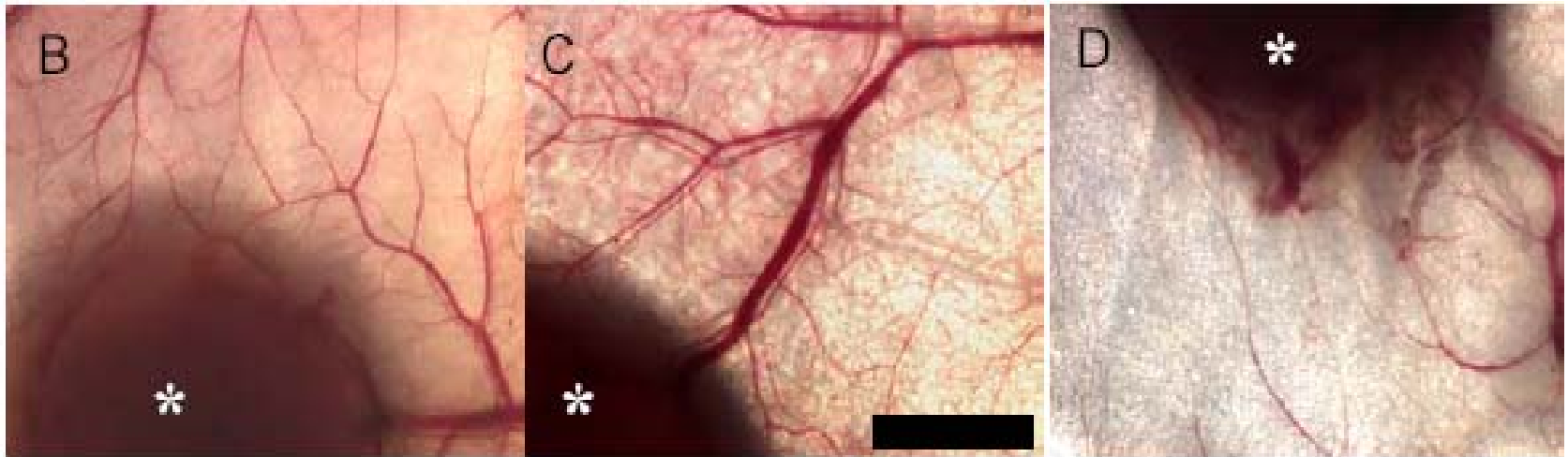
Tumor Neovasculature Inhibition

Blocking Tumor Angiogenesis

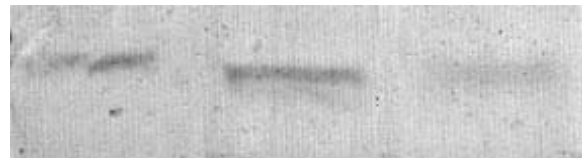
Treatment
Control

siRNA
Control

mVEGFR2
siRNA



mVEGFR2 →



Western Blot

Path to IND

- ❖ **Preclinical Efficacy:** 5 *in vivo* models of angiogenesis (3 tumor models): internal, academic collaborators
- ❖ **Pharmacology:** biodistribution and PK ongoing
- ❖ **Toxicology:** two species, rodent and primate: GLP contractor
- ❖ **Chemistry & Manufacturing (CMC):** cGMP contractor
- ❖ **Phase I protocols:** Clinical collaborator
- ❖ **Regulatory:** Regulatory affairs contractor
- ❖ **pre-IND meeting:** FDA oncology reviewers

Integrated Translation Activities

❖ Pharmacology, Toxicology, and ADME

- Therapeutic Efficacy, Clinical Integration
- Pharmacology Studies (with Tox Supplies)
- Mouse & Monkey GLP Tox/ADME Studies

❖ Chemistry and Manufacturing Controls, Stability

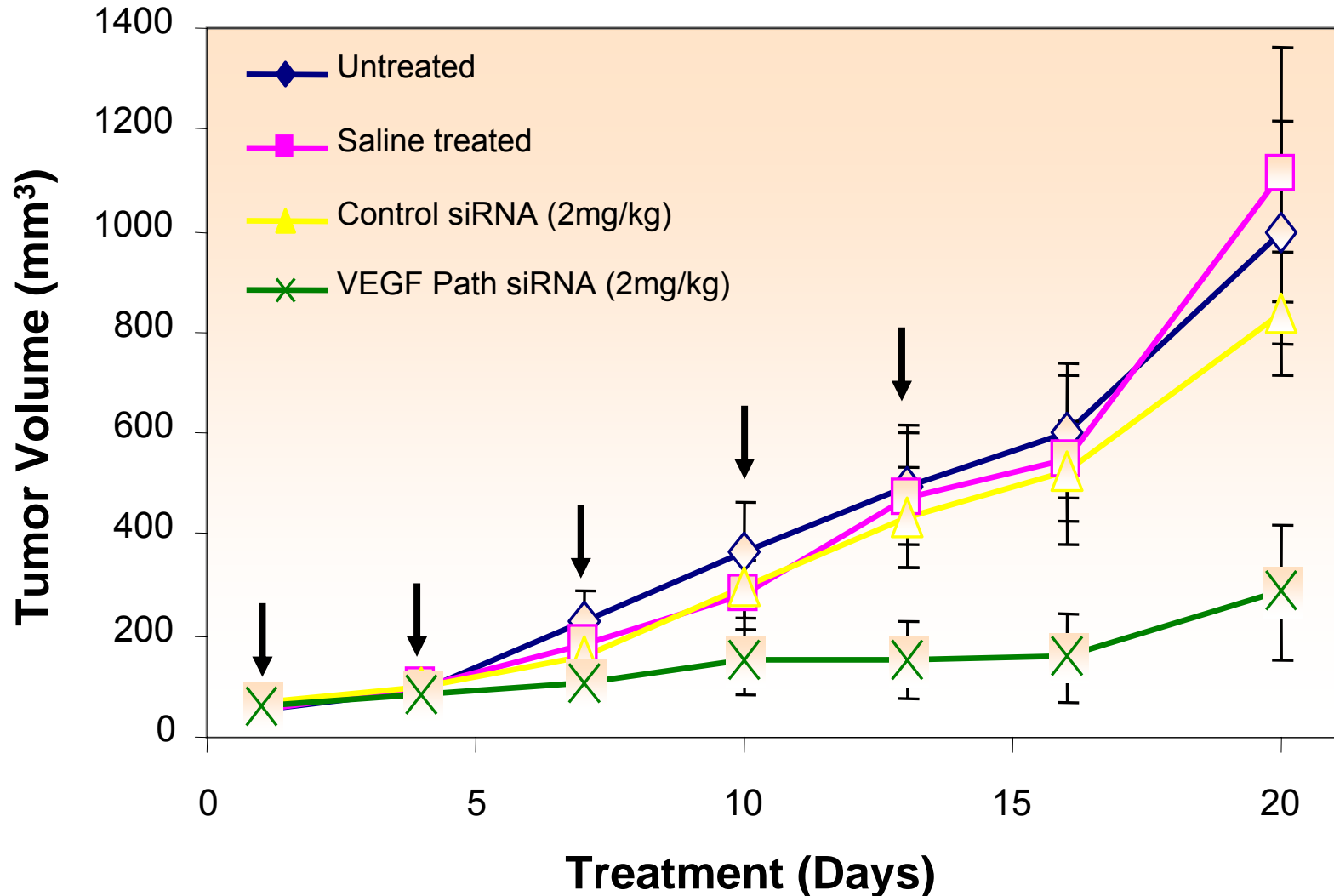
- API & Carrier Manufacturing
- Product Vial Manufacturing
- In Process and QC Assays
- Stability Testing
- Production of GLP Tox and Clinical Supplies

❖ Phase I Clinical

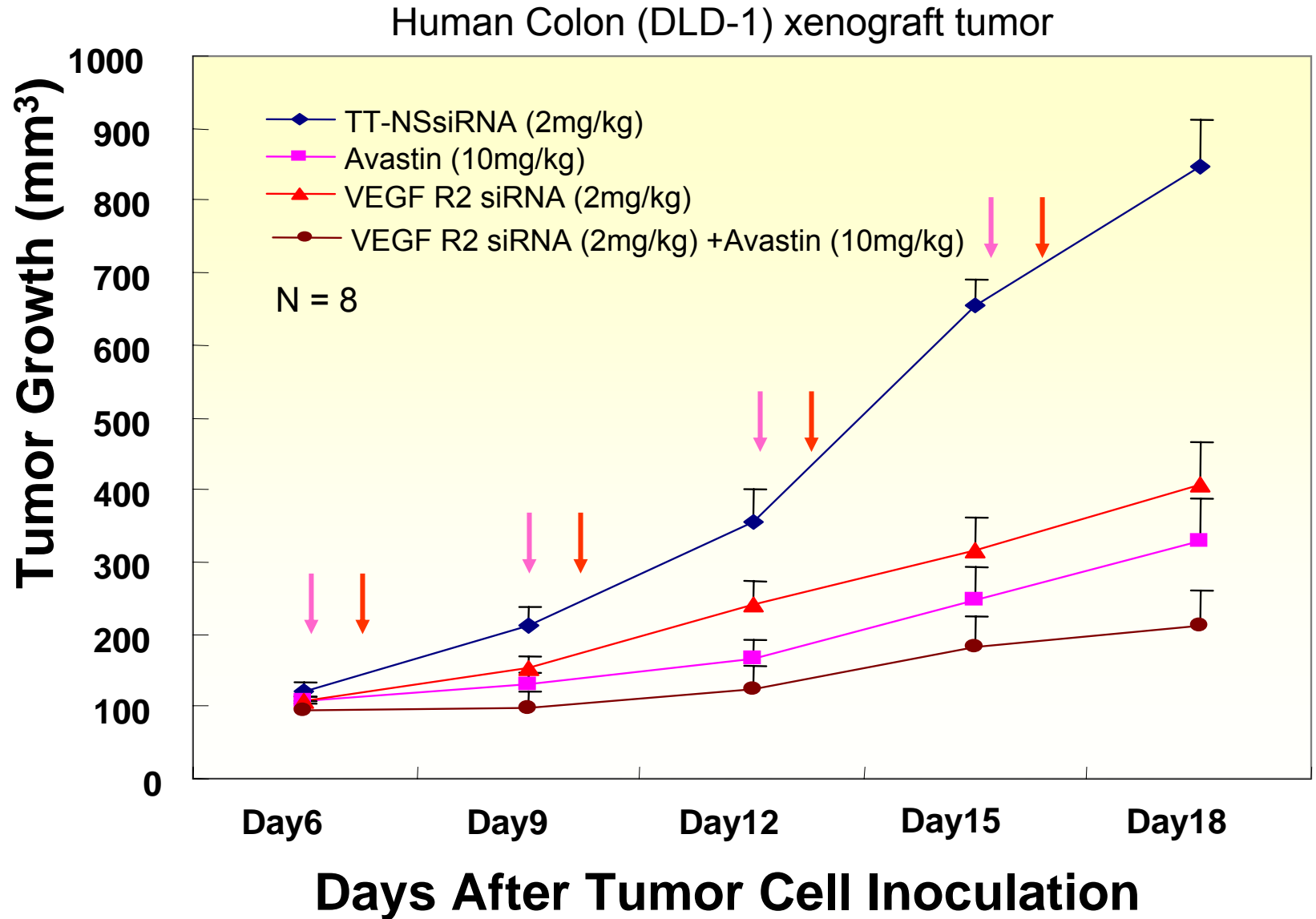
- Clinical Protocol, Case Report Forms
- IND Prep and FDA Interaction

Renal Carcinoma Treatment

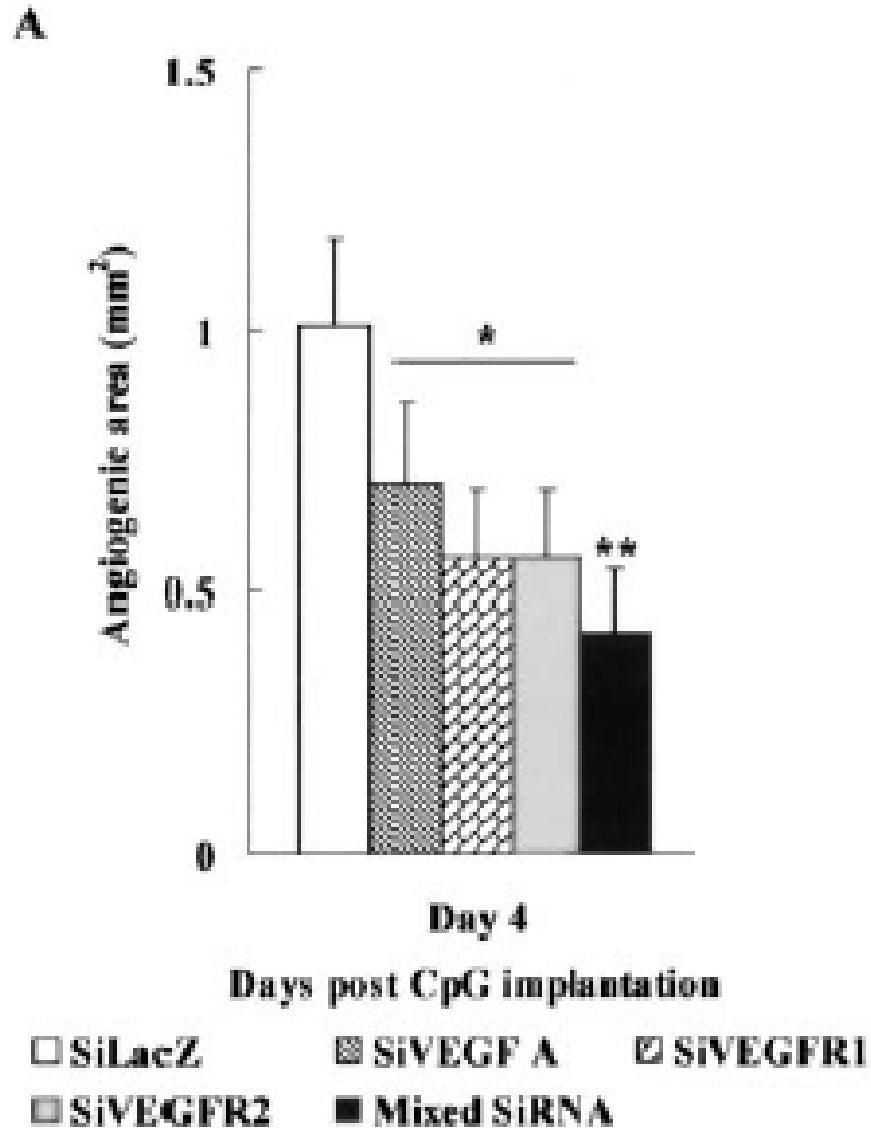
Human Renal (786-O) Xenograft Tumor



siRNA Nanoparticle Combination with Avastin



Sequence Independence & Cocktail Therapy



Integrated Translation Activities

❖ Pharmacology, Toxicology, and ADME

- Therapeutic Efficacy, Clinical Integration
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❖ Chemistry and Manufacturing Controls, Stability

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- In Process and QC Assays
- Stability Testing
- Production of GLP Tox and Clinical Supplies

❖ Phase I Clinical

- Clinical Protocol, Case Report Forms
- IND Prep and FDA Interaction

Self-Assembly Manufacturing

Chemically synthesized
polymer conjugate in water



Chemically synthesized
siRNA in water



Mix



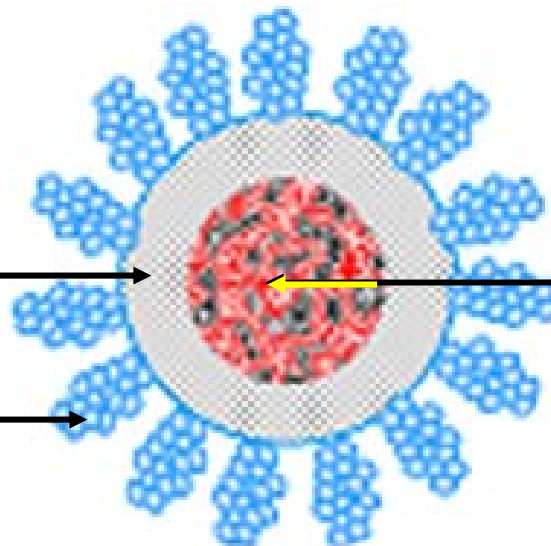
Steric Coat



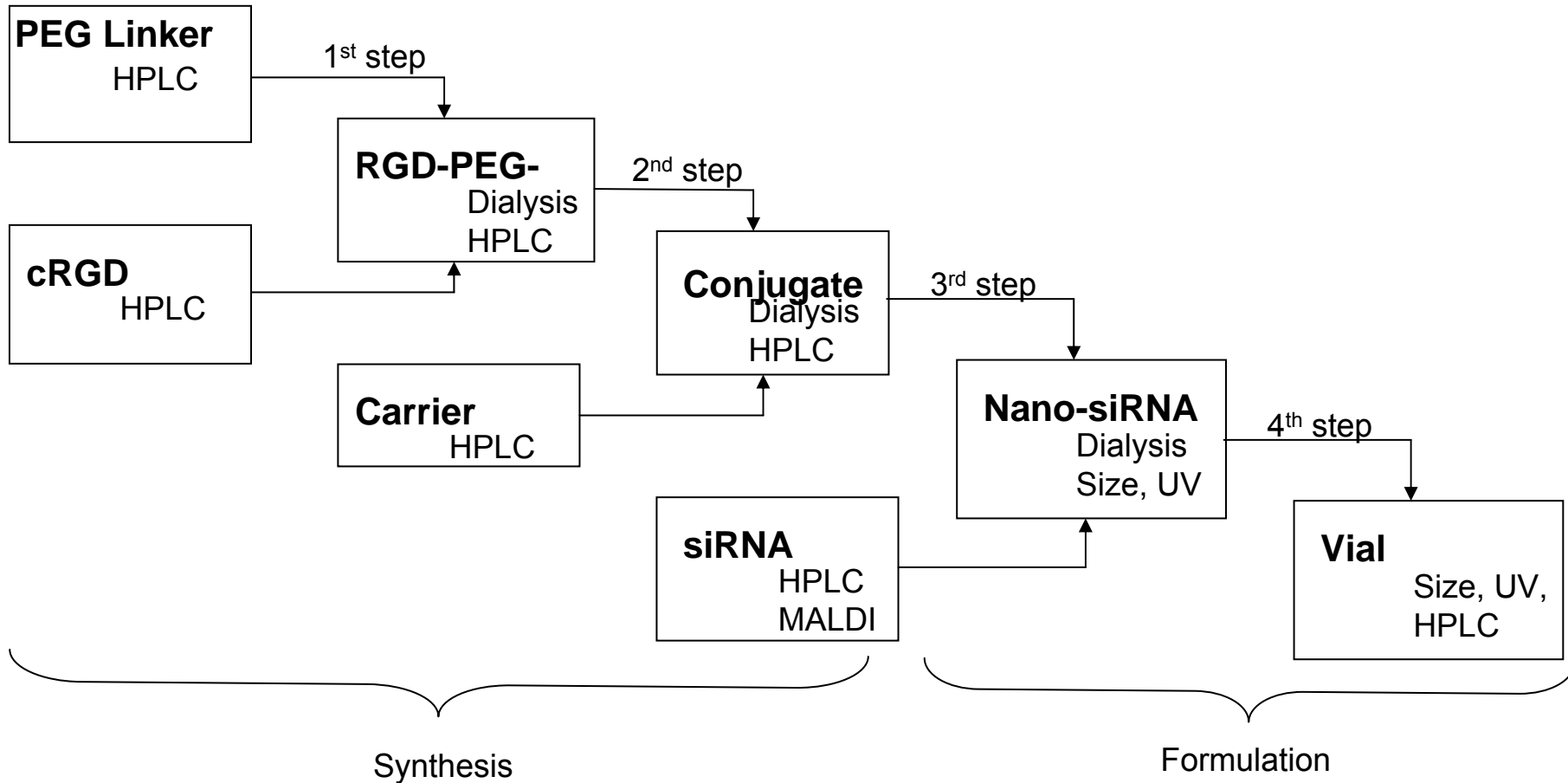
Active Core



RGD Ligand



Synthesis & Manufacturing



Integrated Translation Activities

❖ Pharmacology, Toxicology, and ADME

- Therapeutic Efficacy, Clinical Integration
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❖ Chemistry and Manufacturing Controls, Stability

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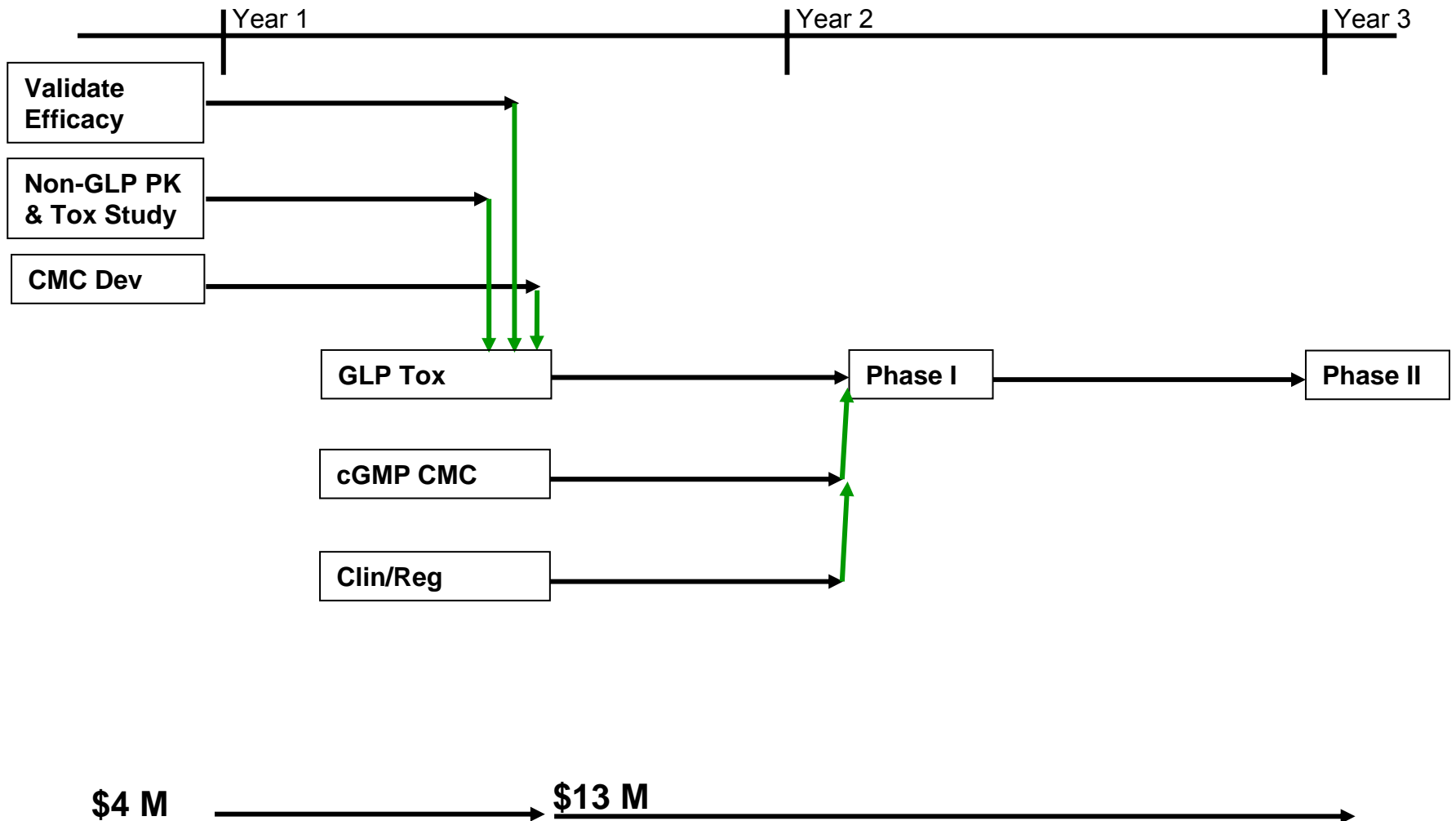
❖ Phase I Clinical

- Clinical Protocol, Case Report Forms
- IND Prep and FDA Interaction

Phase I Draft Plan

1. Weekly schedule.
1 cycle: weekly X3, one week off
2. Approximately 24 patients/site, two sites
3. Inclusion criteria: patients with histologically confirmed solid tumors, tumor progression after receiving standard/approved chemotherapy or where there is no approved therapy, life expectancy of at least 3 months
4. Pharmacokinetic and pharmacodynamic studies
5. PET scans before and after administration in patients at the highest dose to detect initial hints of anti-tumor activity
6. Approximate duration of 9 months

Translation Plan Timing



PreIND Meeting

- ❖ No major regulatory obstacles for “cocktail” Phase I
- ❖ CMC plans adequate for Phase I
 - ✓ API contract cGMP manufacturing
 - ✓ Contract nanoparticle synthesis
 - ✓ Contract manufacturing and analytics
 - ✓ Overlapping stability
- ❖ Pharm/Tox GLP study plans adequate for Phase I
 - ✓ Murine and primates, clotting biology essential
 - ✓ “Empty” nanoparticle group
- ❖ Nano-siRNA cocktail clinical translation is viable

AparnaBio Pipeline – siRNA Nanoparticles

siRNA Nanoparticle Therapeutics

- ABS-393 Cancer Therapeutic
 - Multi-functional Tumor Targeted Nanoparticle Therapeutic
 - Localizes siRNA At Tumor
 - Validated Clinical Mechanisms of Action
 - Strategy applicable to many cancers (NSCLC, colorectal, etc.)
- Follow-on Products
 - Circulating Tumor Cell Targeted Therapeutics
 - Hematological cancers
 - Well Characterized Cell Receptors For Targeting
 - Tumor “Stem Cell” Targeted Therapeutics
 - Circulating Lymphocytes Associated with Inflammatory Disease
 - Preclinical *in vivo* validation with model antibody and siRNA

siRNA Nanoparticles: Next “Antibody” Success

- ❖ Like mAb, many forms with IP “freedom to operate”
- ❖ Like mAb, specific proprietary products
- ❖ But often need combined siRNA and drug delivery:
 - Targeted nanoparticles solving siRNA delivery
 - AparnaBio has therapeutic solutions

Acknowledgement

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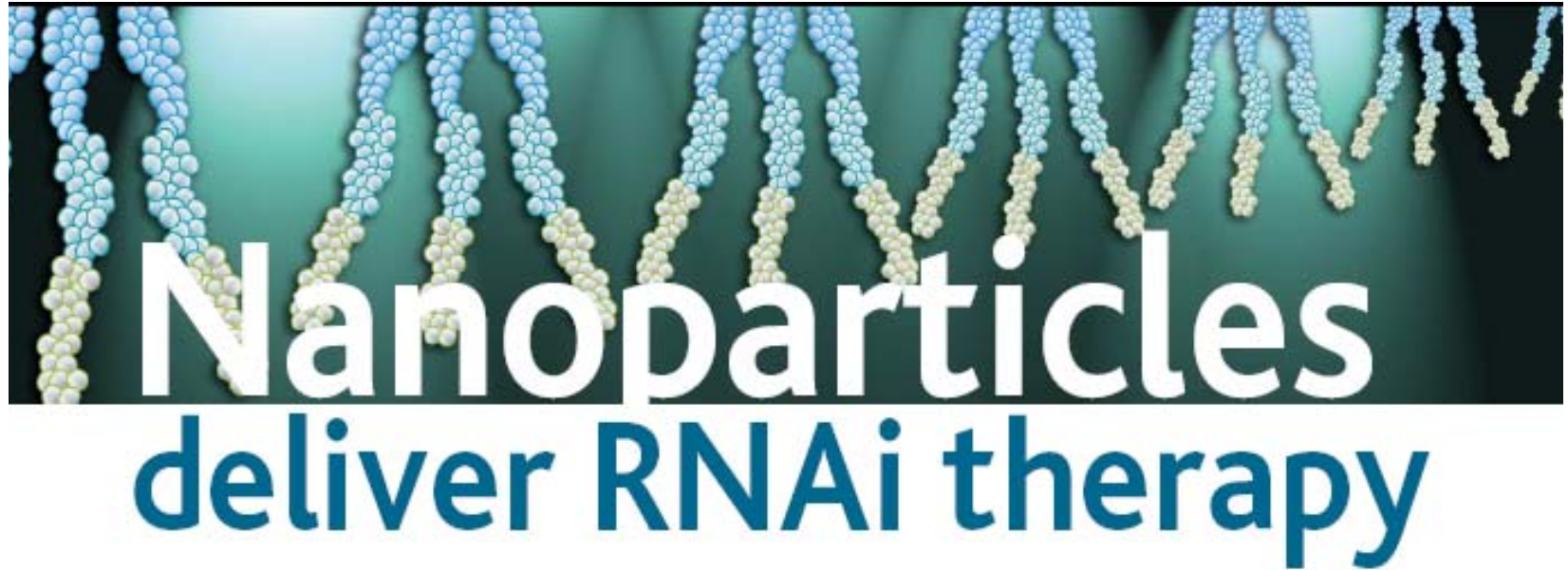
Dr. Walter Tian

Invitrogen

Dr. Tod Woolf

Dr. Dmitry Samarsky

Tissue Targeting With Nanoparticles



Nanoparticles deliver RNAi therapy

by Martin C. Woodle* and Patrick Y. Lu

Nanotechnology-based advanced materials are rapidly expanding development of better medicines. Long-standing efforts with lipid and polymer colloidal delivery systems, i.e. nanoparticles, have yielded better imaging and therapy. These benefits of

The application of nanotechnology has tremendous potential in healthcare, particularly for the development of better pharmaceuticals. Great strides are already being made in creating nanoparticle systems for targeted drug delivery. Nanoenabled drug delivery has already been