Biological Nanotechnology:

Applying Interdisciplinary Nanotechnology for the Treatment of Cancer

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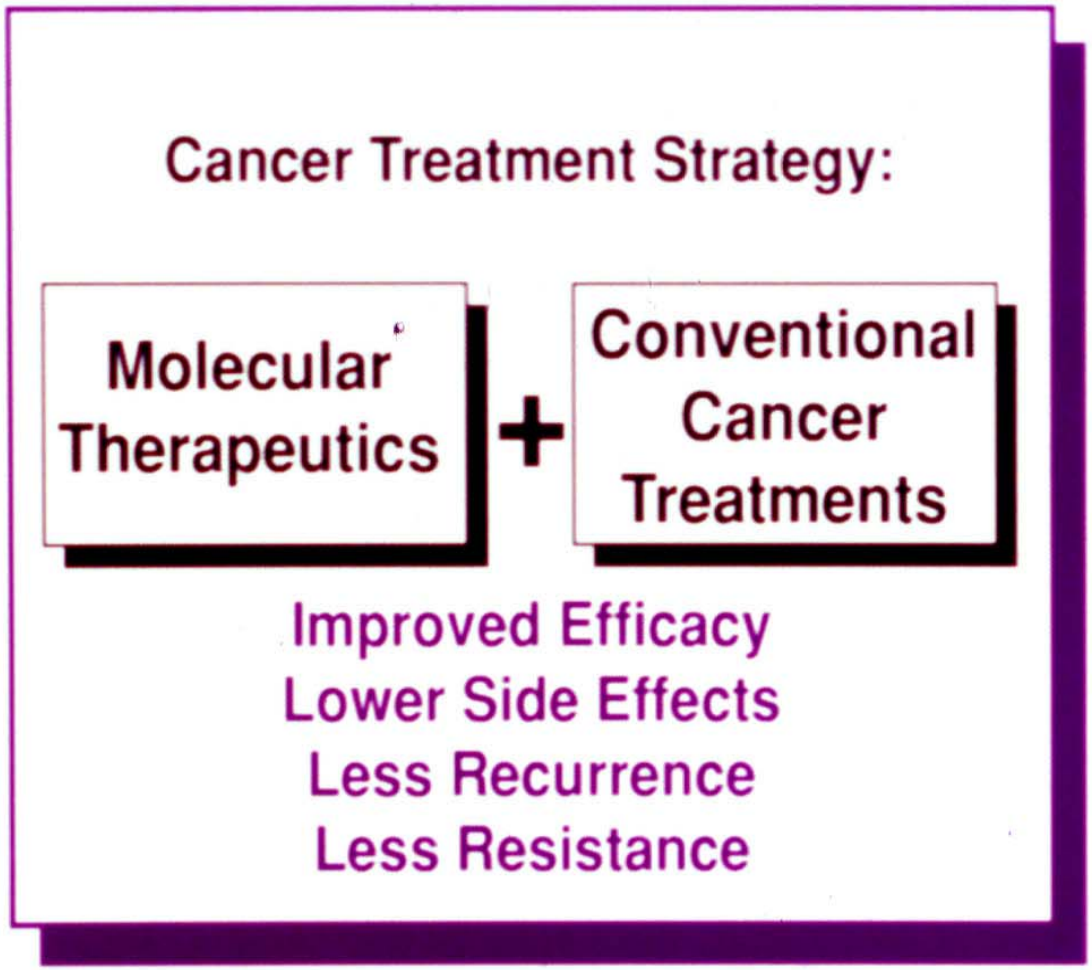
Seeking Synergy in Cancer Treatment:
The Whole is Greater than the Sum of the Parts



SynerGene Therapeutics, Inc.

Synergy in Cancer Treatment by Bringing the Pieces Together

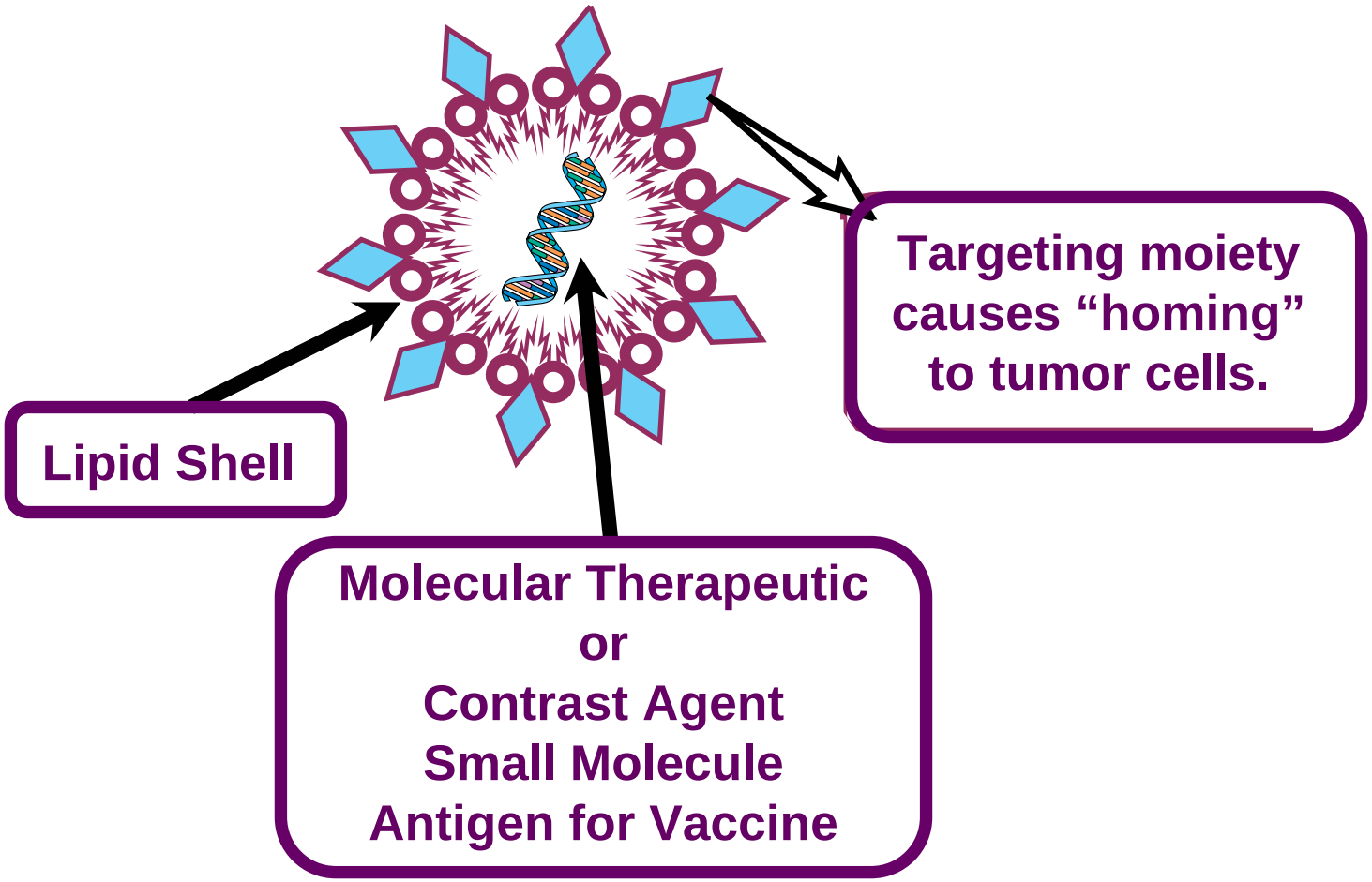
Nanotechnology	+	Molecular Therapeutics	+	Conventional Therapeutics
Nanocapsular Tumor-Targeted Systemic Delivery		Gene Therapy siRNA Therapy Antisense Therapy Small Molecule Therapeutics		Chemotherapy Radiotherapy

The whole can be greater than the sum of the parts!

Characteristics of an Ideal Cancer Therapeutic

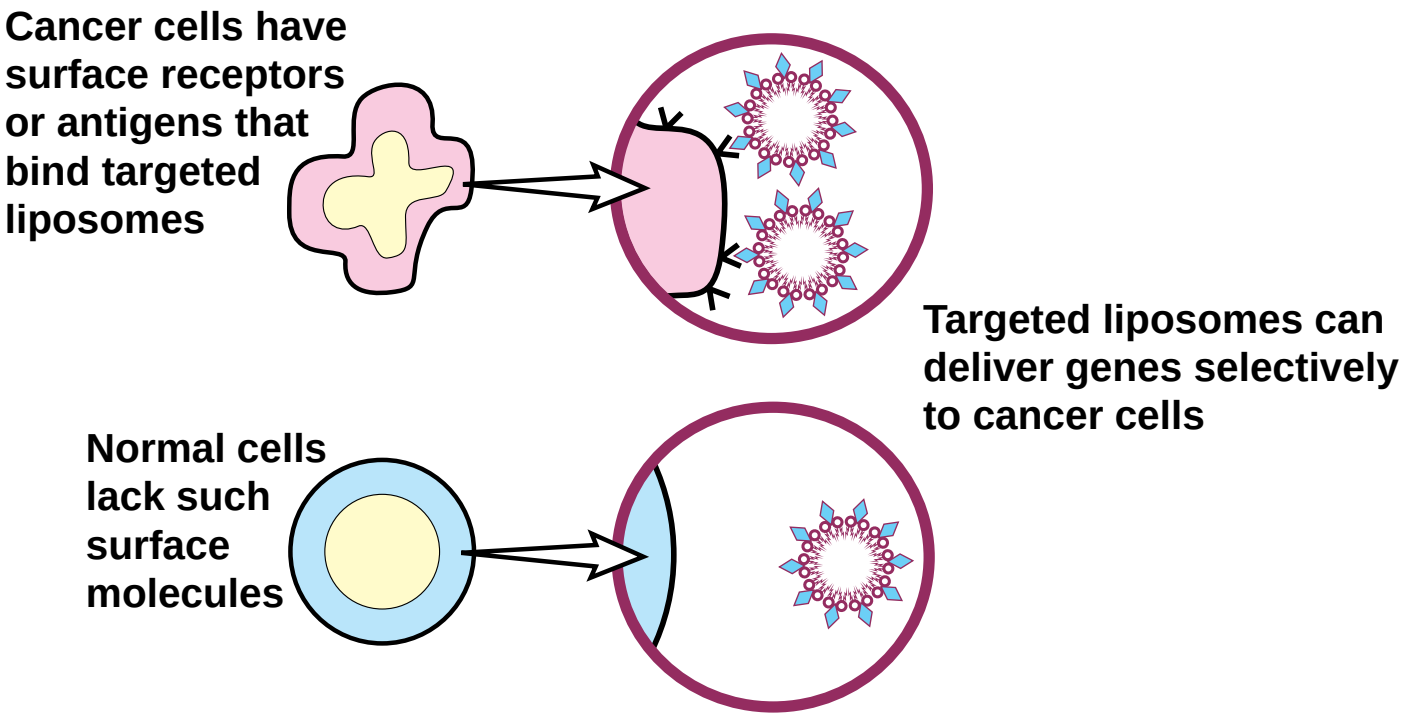
- **Is selectively delivered to tumor cells thereby minimizing side effects on normal cells**
- **Is capable of reaching not only the primary tumor but also distant metastases (that end up killing patients)**
- **Is effective at killing the tumor cells it reaches**

Tumor-targeted Nanocapsules



Selective Targeting of Tumors by Nanocapsules

Targeted Liposomes Can Distinguish Cancer Cells



Ligand Targeting

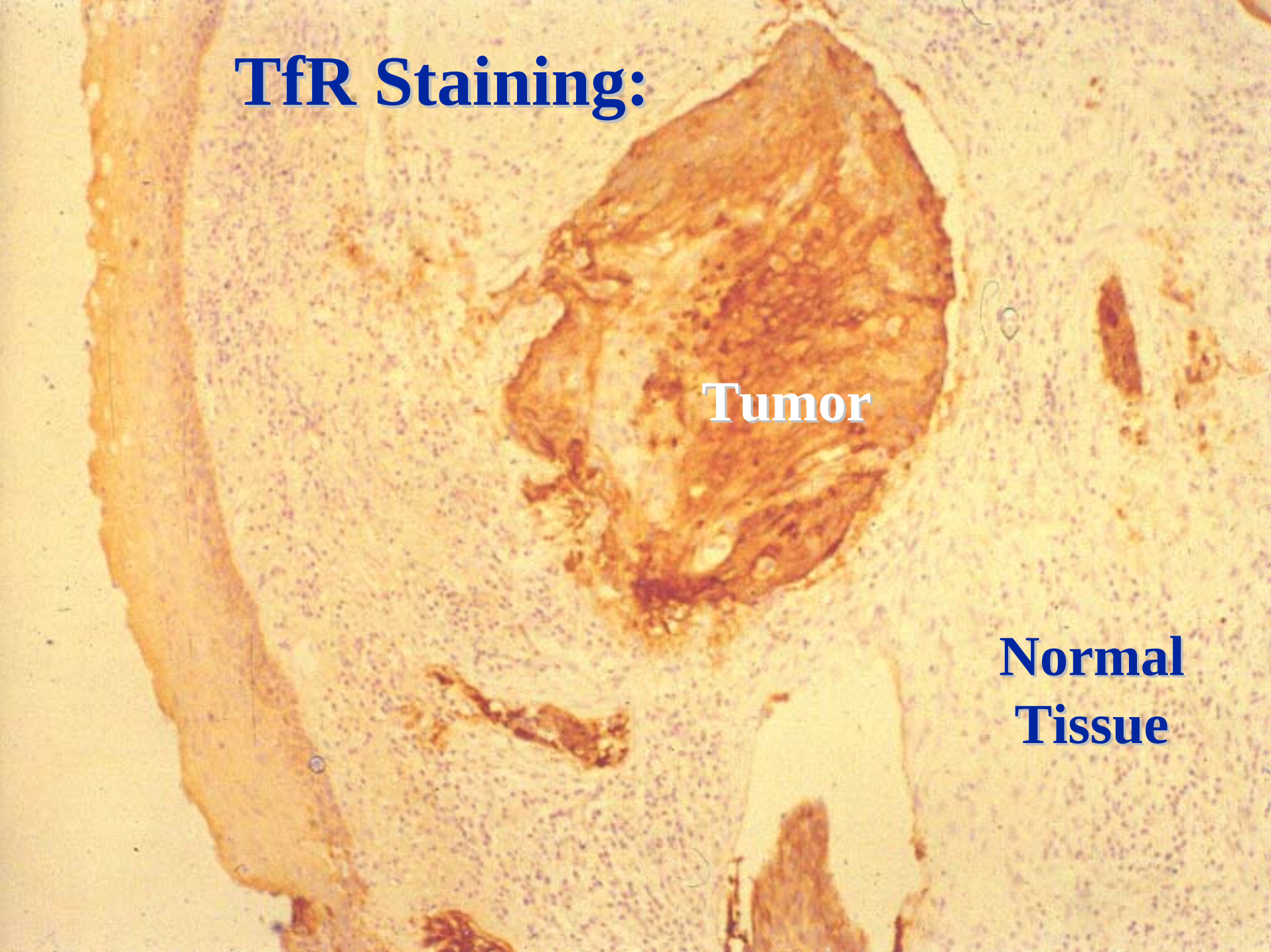
Folate

- Receptor elevated in many tumor types
- Internalized by receptor-mediated endocytosis

Transferrin

- Receptor elevated in many tumor types
- Internalized by receptor-mediated endocytosis
- Receptor levels correlate with proliferative ability

TfR Staining:



Features Required for a Gene Therapy Approach to Killing Tumor Cells *in vivo*

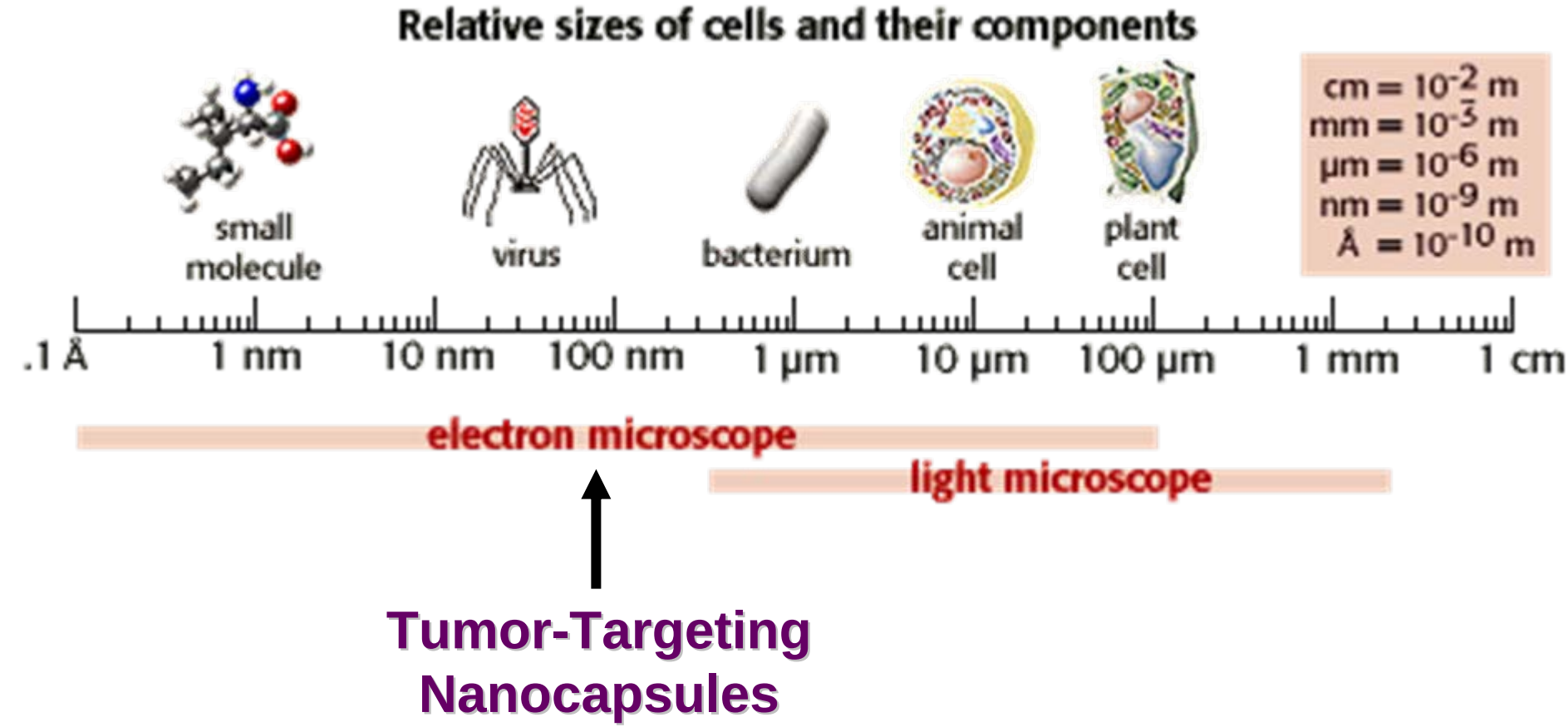
1. Selective delivery of the plasmid encoding the gene to tumor cells

2. Expression of the gene delivered in the tumor cells (the more the merrier or efficacious “bystander effect”)

3. Gene product either kills cells in which it is expressed or makes these cells more susceptible to killing by another agent

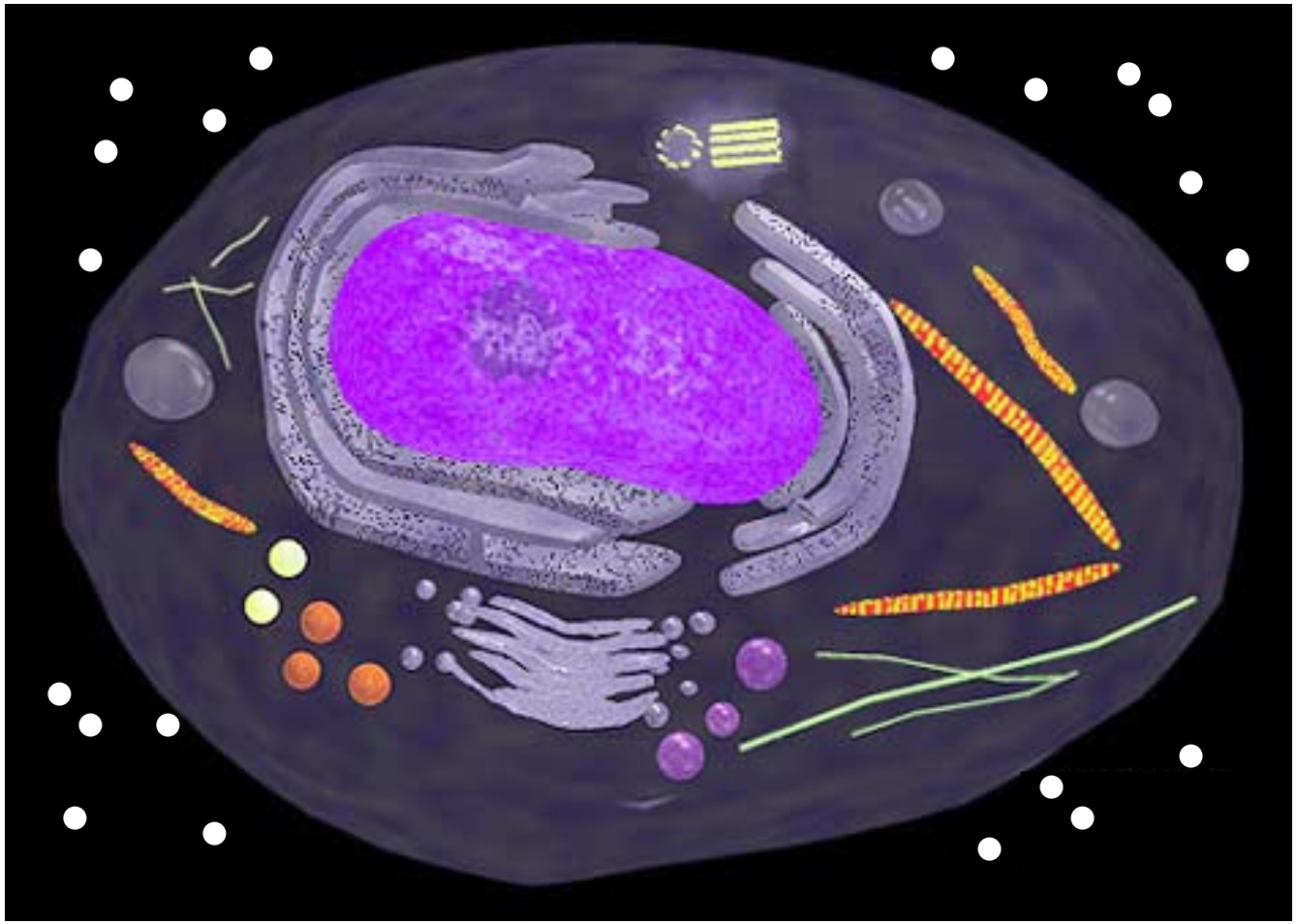
Relative Sizes of Things

If a cell of 10µm were as wide as this slide, this bar would represents a 100nm nanoparticle: -

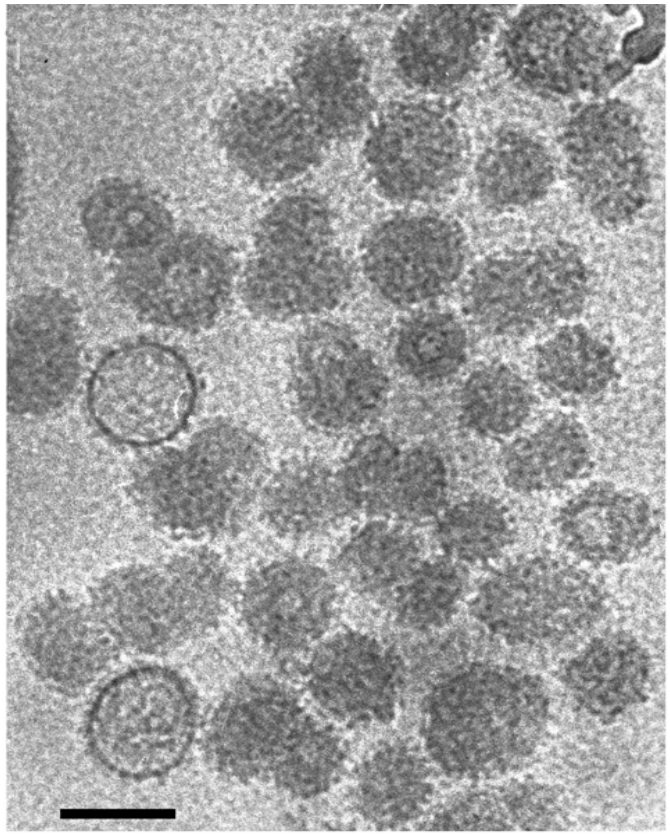


Tumor-Targeting Nanocapsules

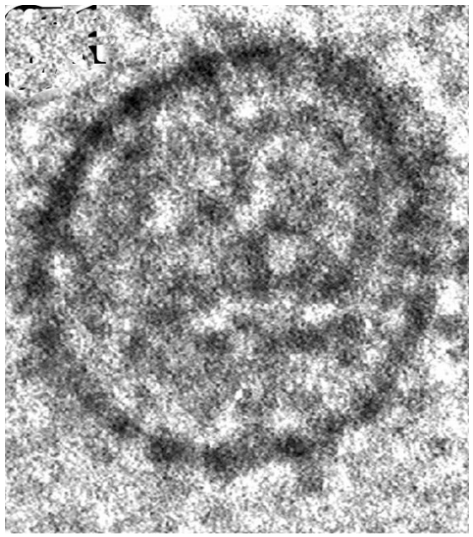
Cell Surrounded by 100nm Nanocapsules

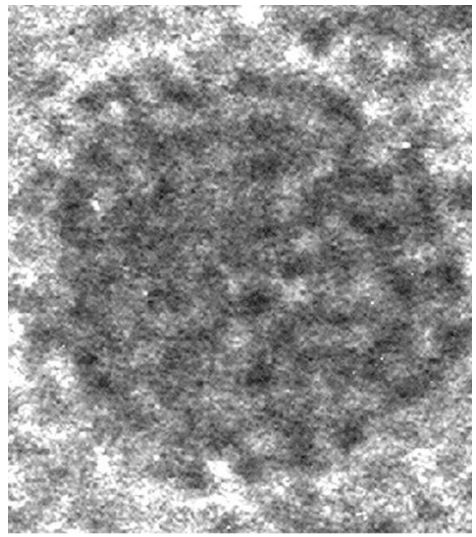


Tumor-Targeted Nanocapsules



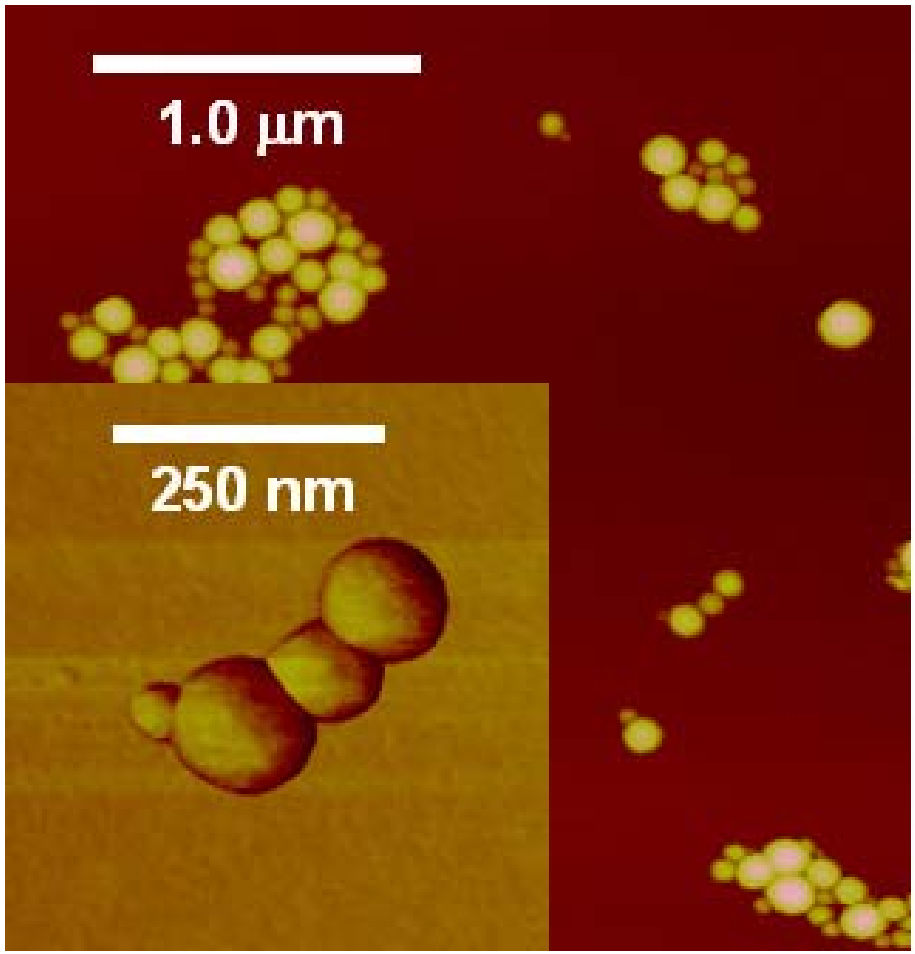
100 nm



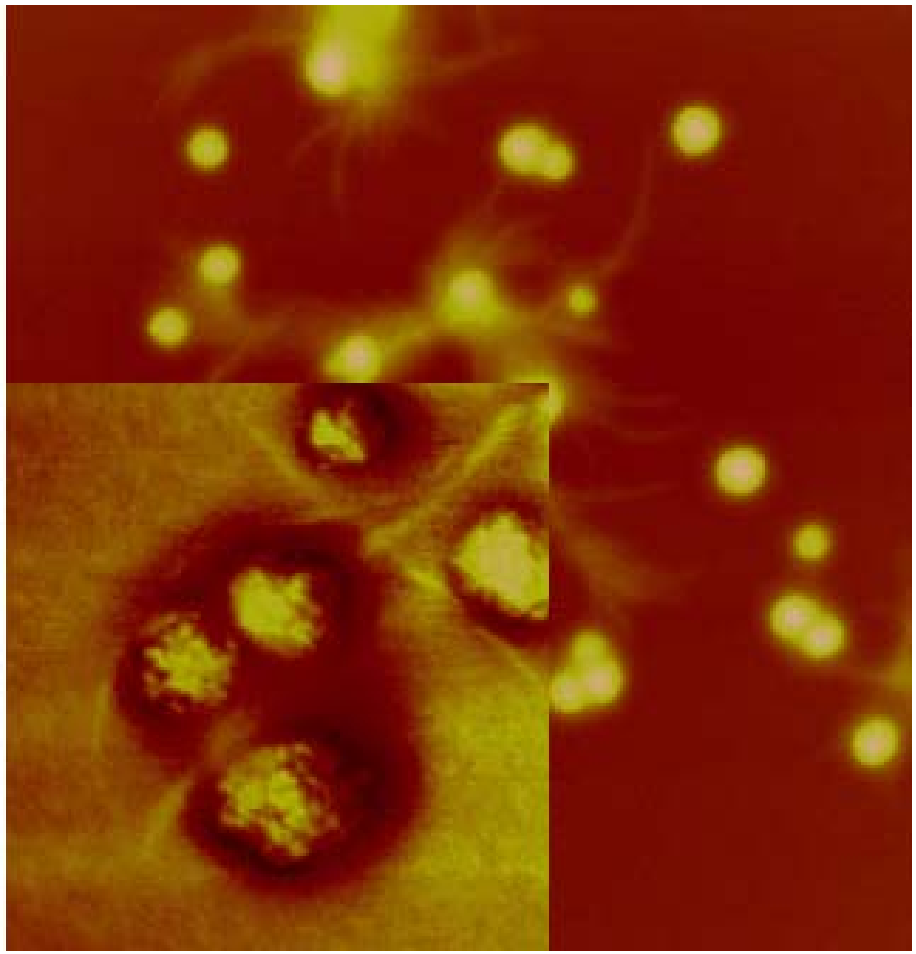


Higher magnification of “virus-like” nanocapsules

Topographical and Phase SPM Images of Nanoimmunocomplexed siRNA



TfRscFv-LipA-siRNA



TfRscFv-LipA-HoKC-siRNA

The inserts represents the magnified Phase contrast image. The scales are the same in each panel

ANIMAL TUMOR MODELS

Mouse Xenograft (Subcutaneous, Orthotopic, Intracranial, or Metastatic)

- Head and Neck (Targeting/Efficacy) Sub, Ortho
- Prostate (Targeting/Efficacy) Sub, Ortho
- Breast (Targeting/Efficacy) Sub, Metas
- Pancreatic (Targeting/Efficacy) Sub, Ortho
- Glioblastoma (Targeting) Sub, IntraC
- Hepatic (Targeting) Sub
- Bladder (Targeting/Efficacy) Sub, Ortho
- Cervical (Efficacy) Sub

Mouse Syngeneic

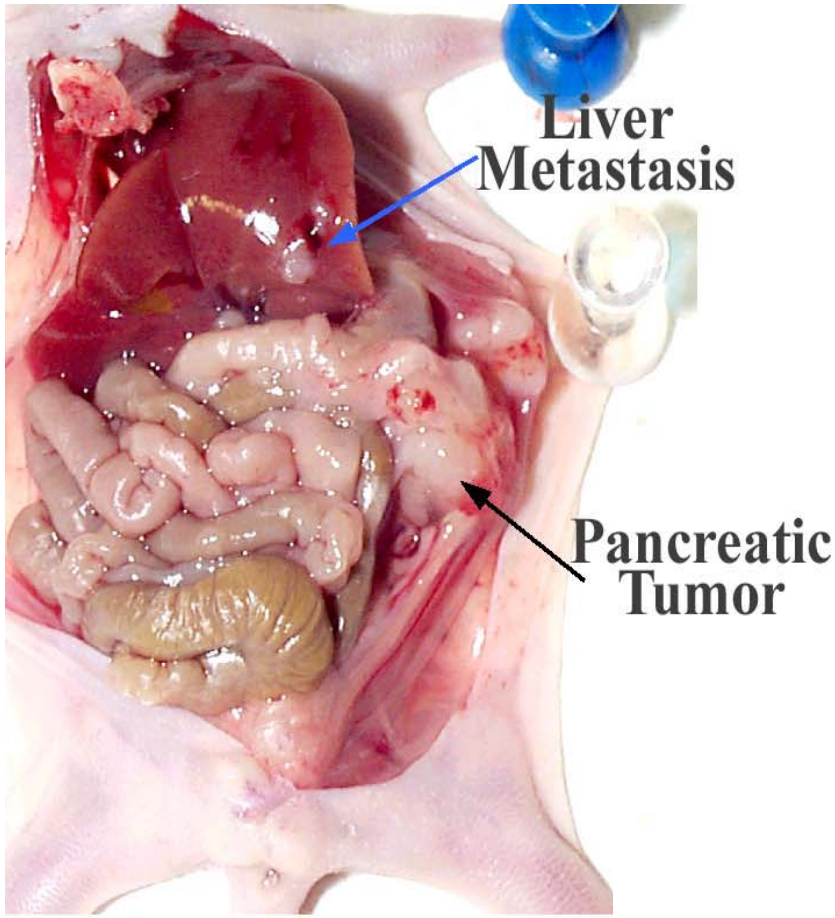
- Melanoma (Targeting/Efficacy) Metas
- Breast (Targeting/Efficacy) Metas

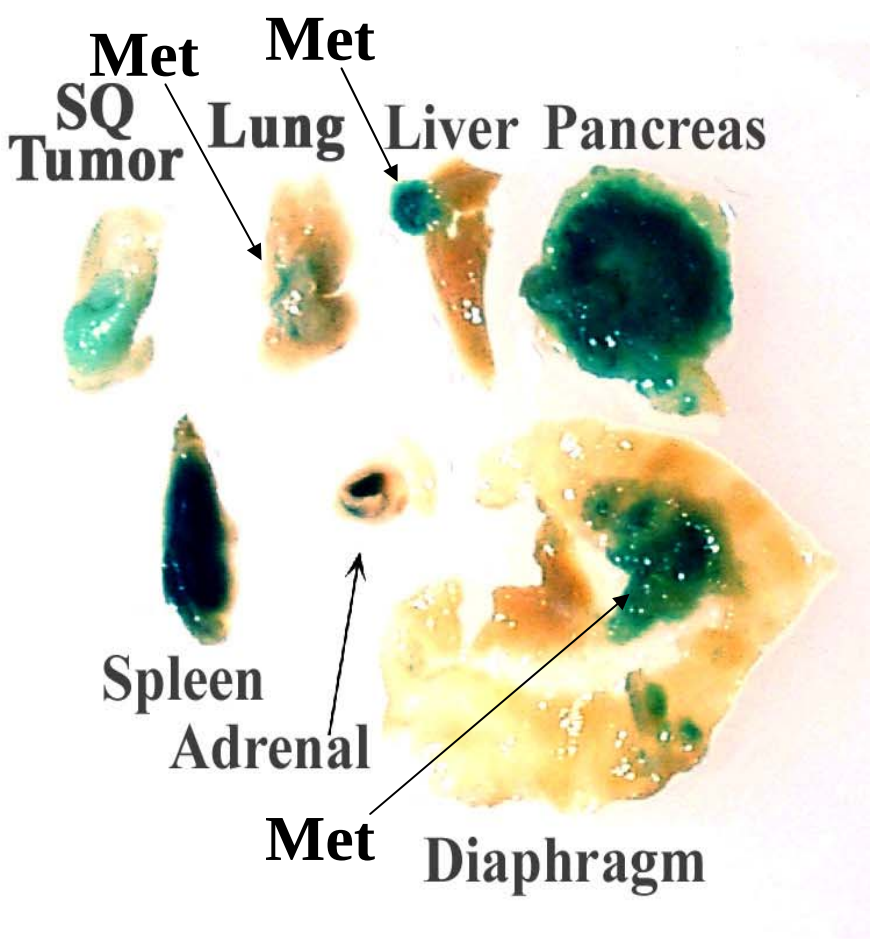
Rat Syngeneic

- Glioblastoma (Targeting/Efficacy) IntraC

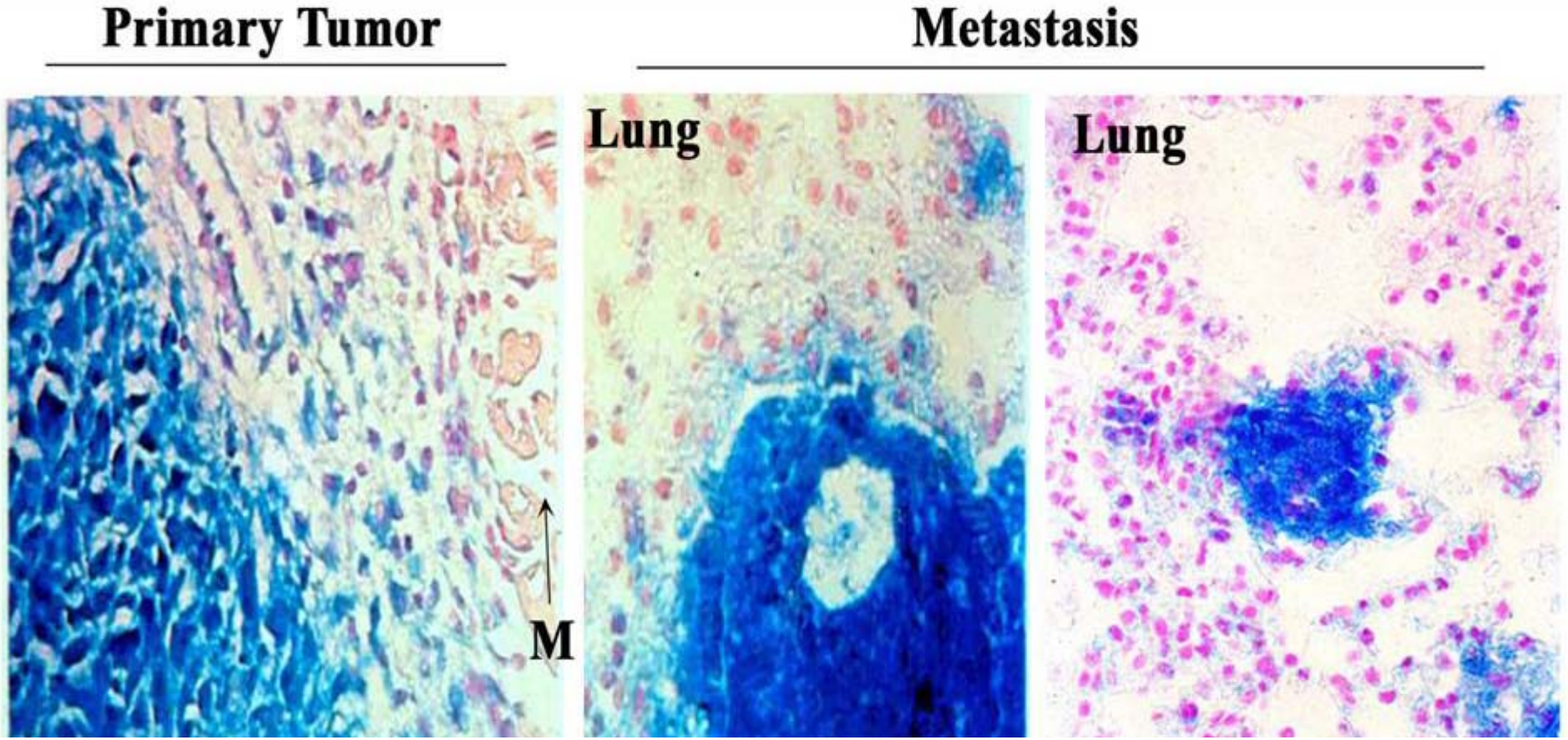
Systemic Delivery of siRNA *via* Nanocapsules

Lac-Z Gene Targeted to Primary and Metastatic Pancreatic Cancer Cells





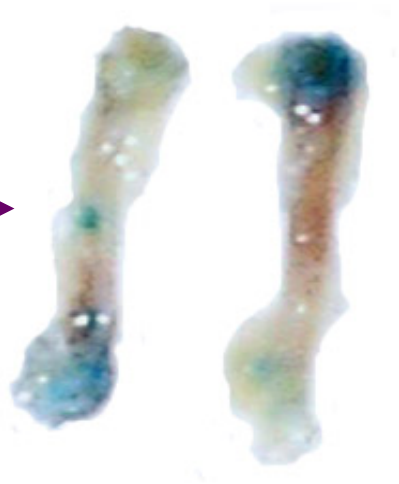
Targeted Delivery of the Lac-Z Gene to Primary and Metastatic Breast Cancer Cells



Demonstration of Tumor Specific Transfection Efficiency of a Systemically Delivered LacZ Gene into Bone Mets in a Prostate Cancer Mouse Model

Mouse Femurs With Prostate Cancer Mets

Met →



Penetrating the Blood Brain Barrier

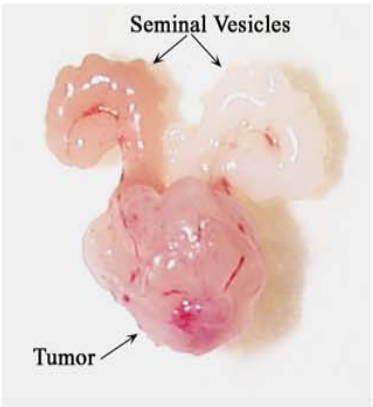
Demonstration of Brain Tumor Specific Transfection Efficiency of a Systemically Delivered Complex Carrying a Gene Encoding for Green Fluorescence Protein (GFP) in a Rat Intracranial Glioma (C-6)

Normal Brain **Tumor**

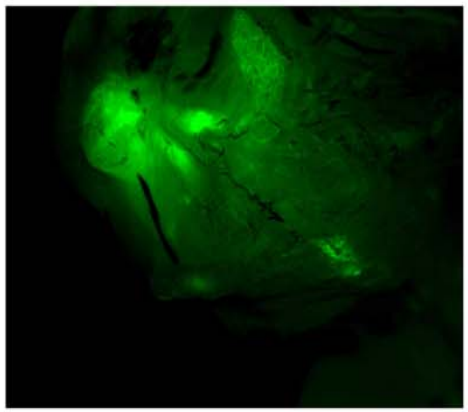
Systemic Delivery of siRNA *via* Nanocapsules

Tumor Targeted Systemic Delivery of Fluorescent Labeled siRNA (nonsense 21 mer) in an Orthotopic Human Prostate Xenograft Mouse Model

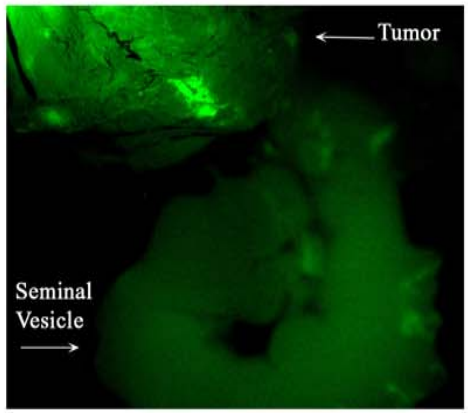
Tumor/Seminal Vesicles



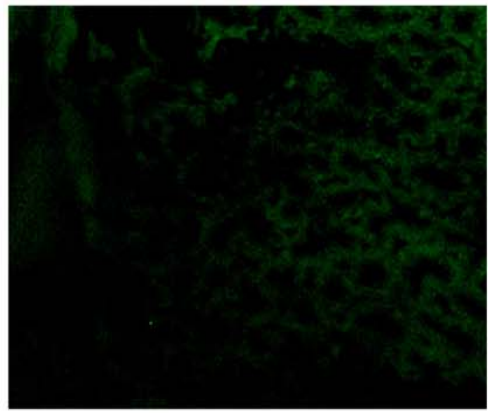
Tumor



Tumor/Seminal Vesicle

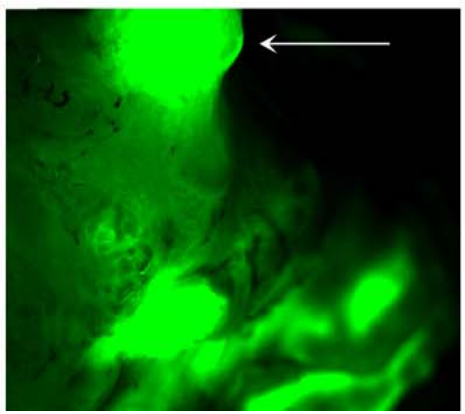


Liver

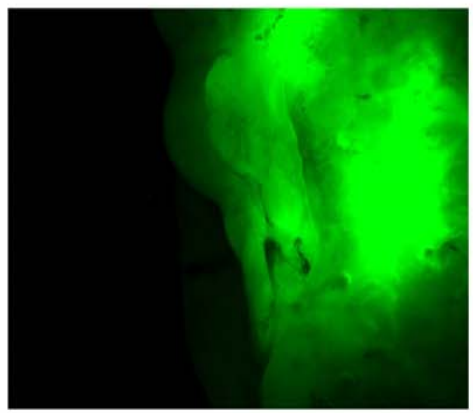


Tumor

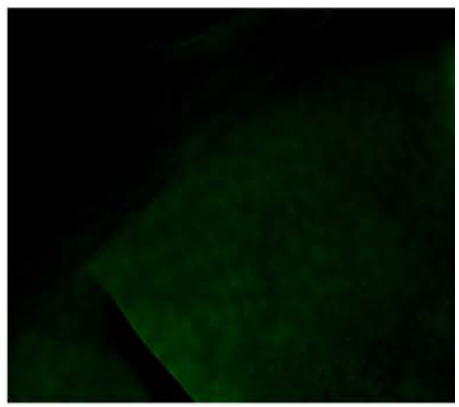
Tumor



Tumor Cross Section

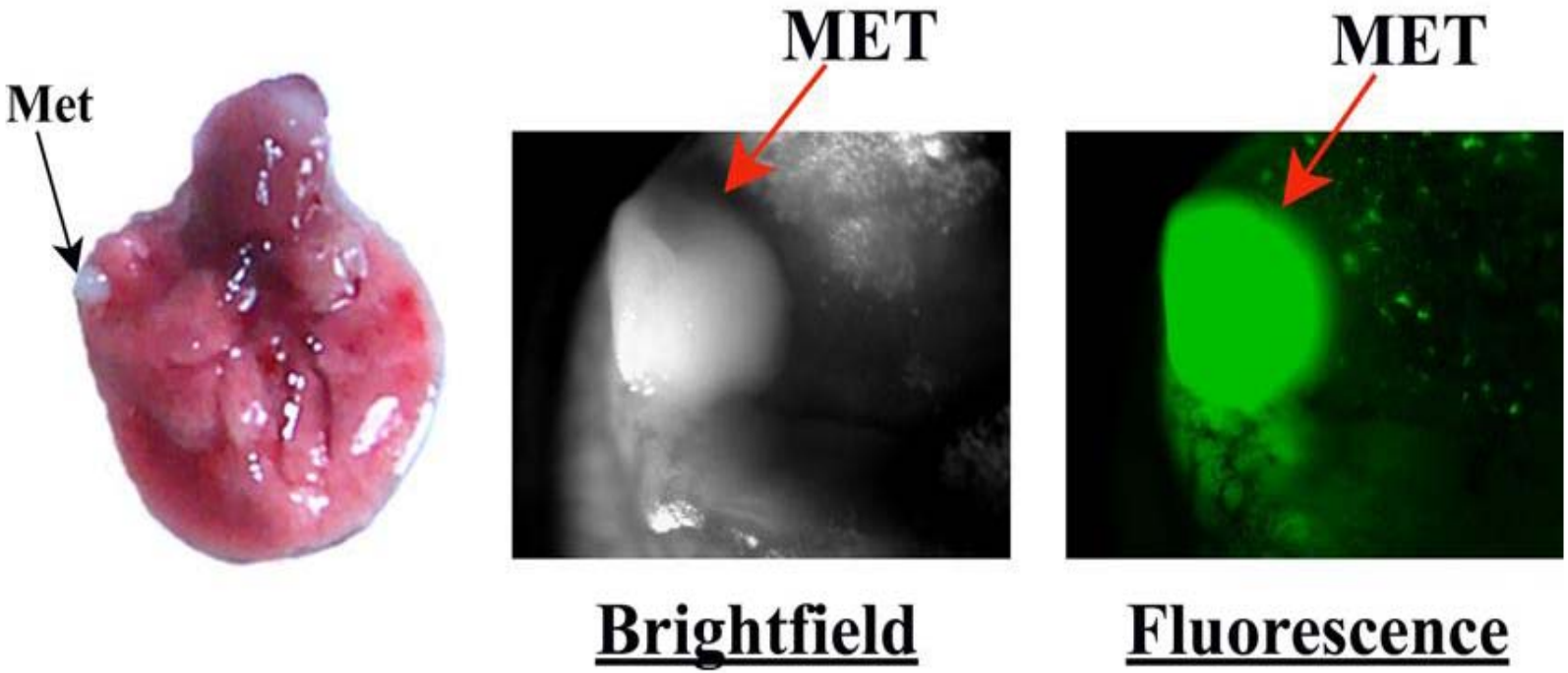


Liver



TriLink Biotechnologies

Delivery of Fl-siRNA to Metastatic Breast Cancer Cells



Synergy in Cancer Treatment by Bringing the Pieces Together

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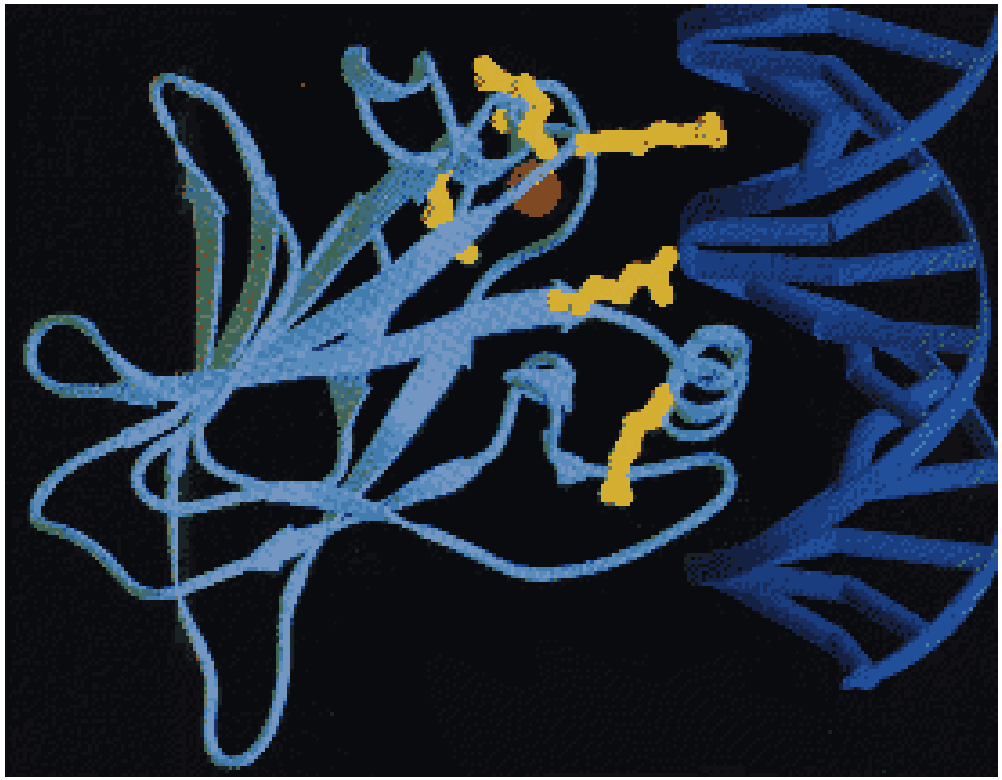
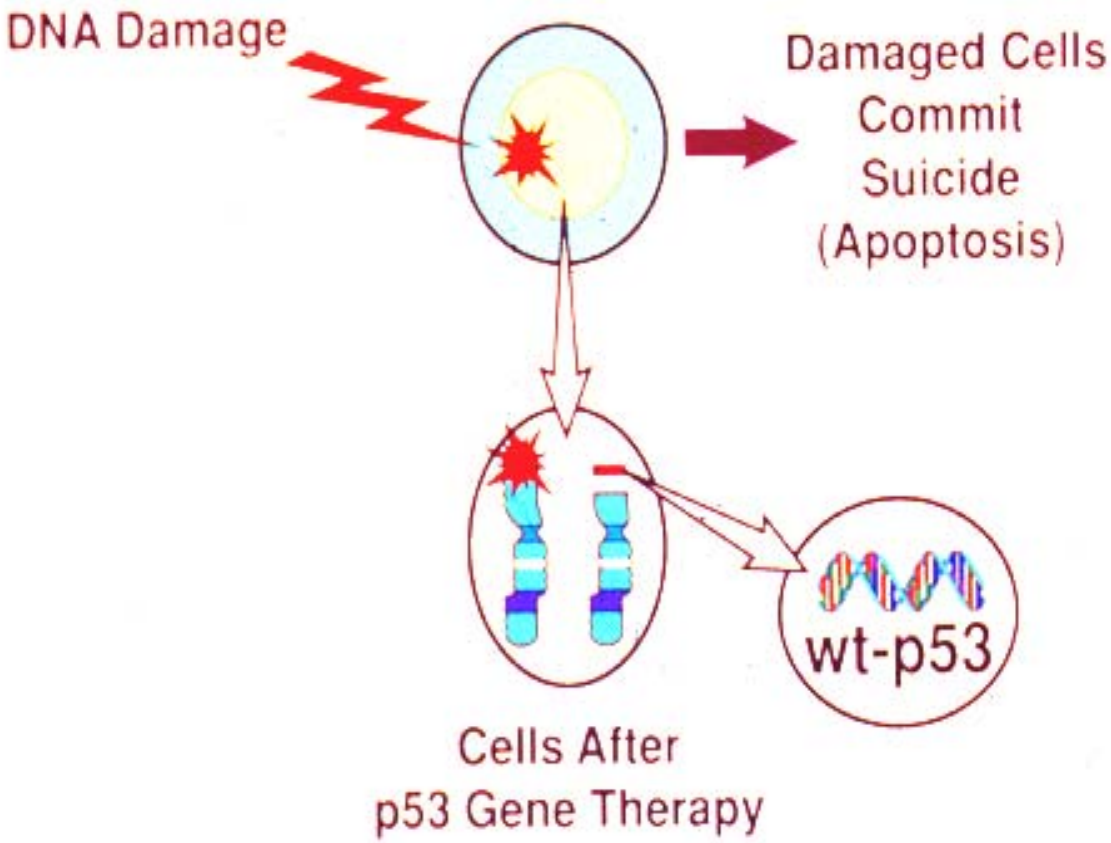
The whole can be greater than the sum of the parts!

SynerGene's First Gene Therapy Target: The Tumor Suppressor p53

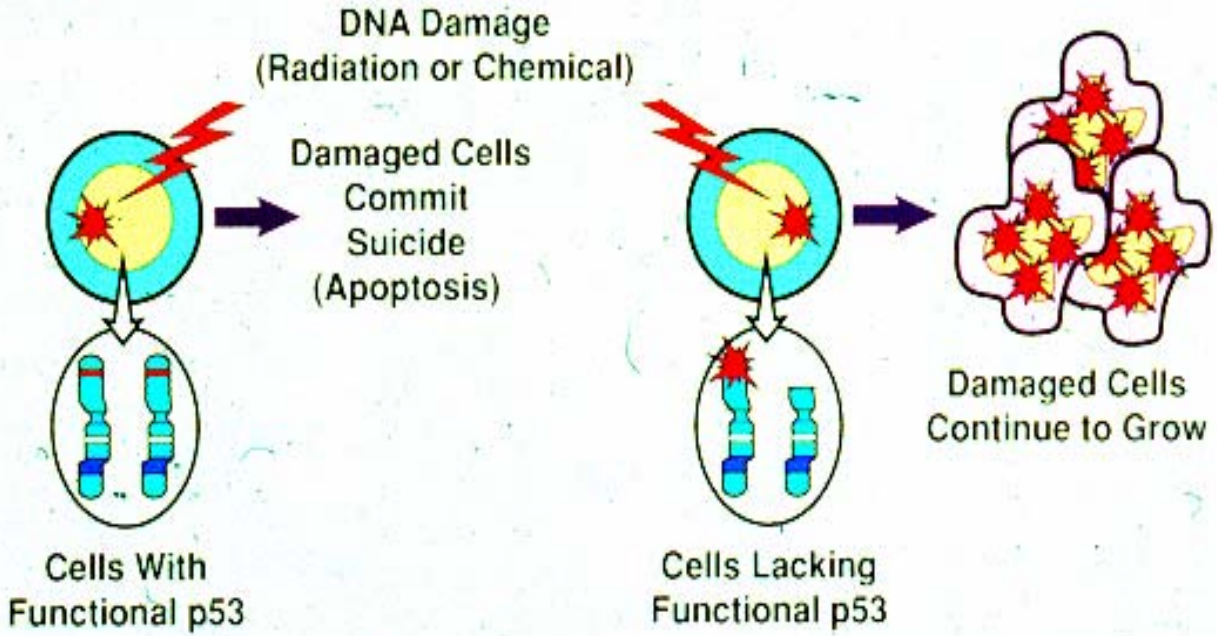


P53 protein (on the left) is shown interacting with both the major and minor grooves of the DNA helix (on the right).

p53 Gene Therapy Restores Sensitivity to DNA Damage

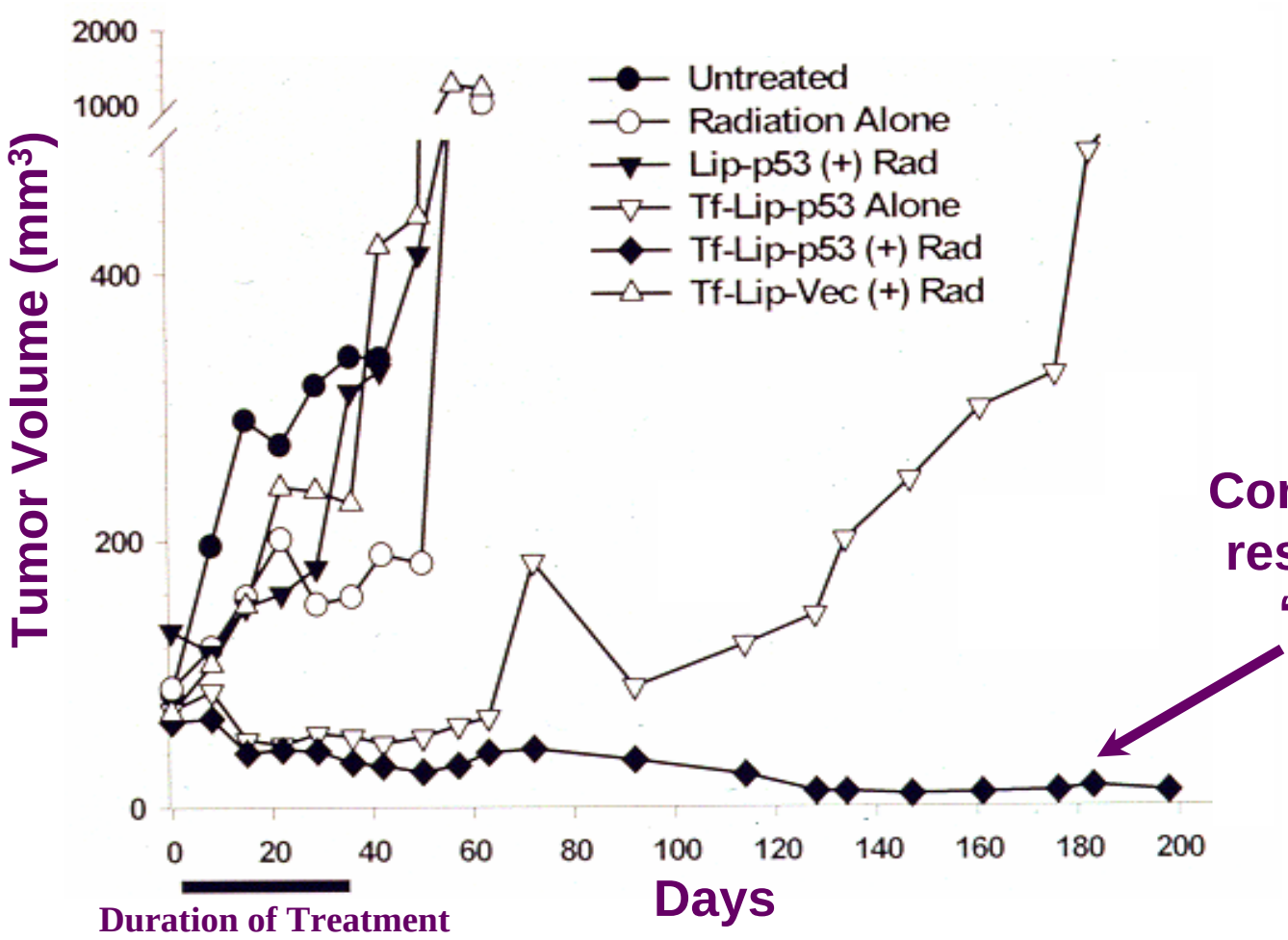


Loss of Functional p53 Confers Resistance to DNA Damage



Radiosensitization

Systemic P53 Gene Delivery by Nanocapsules to Prostate Cancer Cells

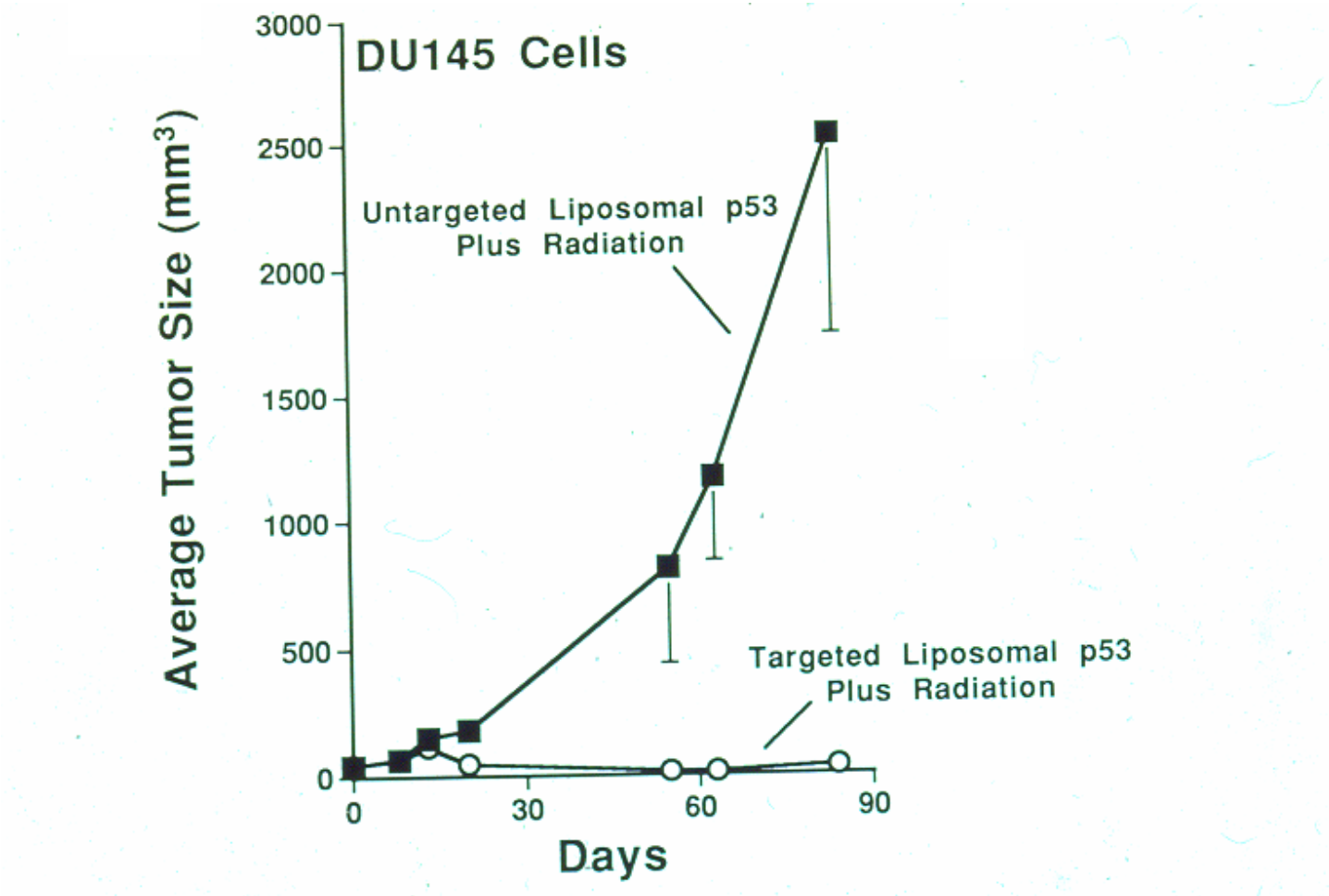


Combination therapy results in long-term "cure" for mice

Mice Bearing Human Prostate Tumors Treated with Nanocapsules Carrying P53

Untreated	Radiation Alone	Targeted P53 Gene Alone	Targeted P53 Gene + Radiation

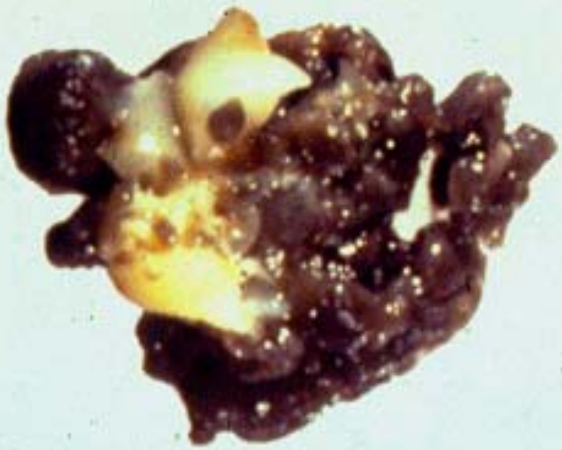
The Importance of Targeting the Nanocapsules



Chemosensitization

Mouse Melanoma Lung Metastasis Model

Untreated

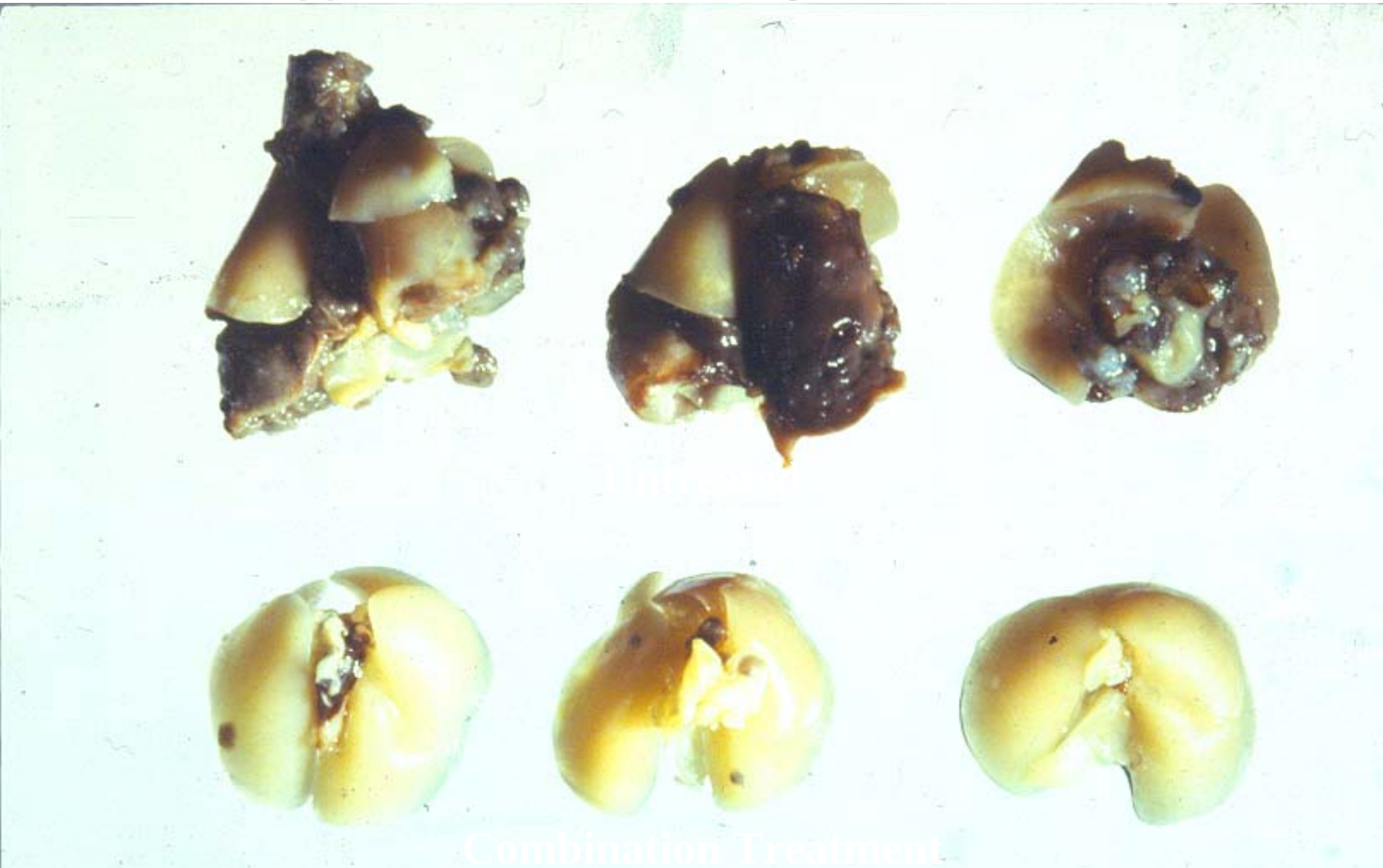


CDDP only

LipAe-p53 plus CDDP

Tf-LipAe-p53 plus CDDP

Lack of Tumor Growth After the Combination of Systemic Normal Tumor Suppressor (p53) Gene Therapy and Chemotherapy in a Melanoma Lung Cancer Metastasis Model



Clinical Relevance

- Combination treatment with the systemic, tumor targeted nanocomplex carrying a molecular therapeutic agent and a conventional therapy would be effective for primary and metastatic tumors.

- Sensitization of tumors to radiation and chemotherapy could result in lowering the effective dose, thereby lessening the severe side effects or decreasing the probability of recurrence.

- Improved delivery and uptake of contrast agents to tumors could lead to earlier detection and treatment, resulting in better outcome.

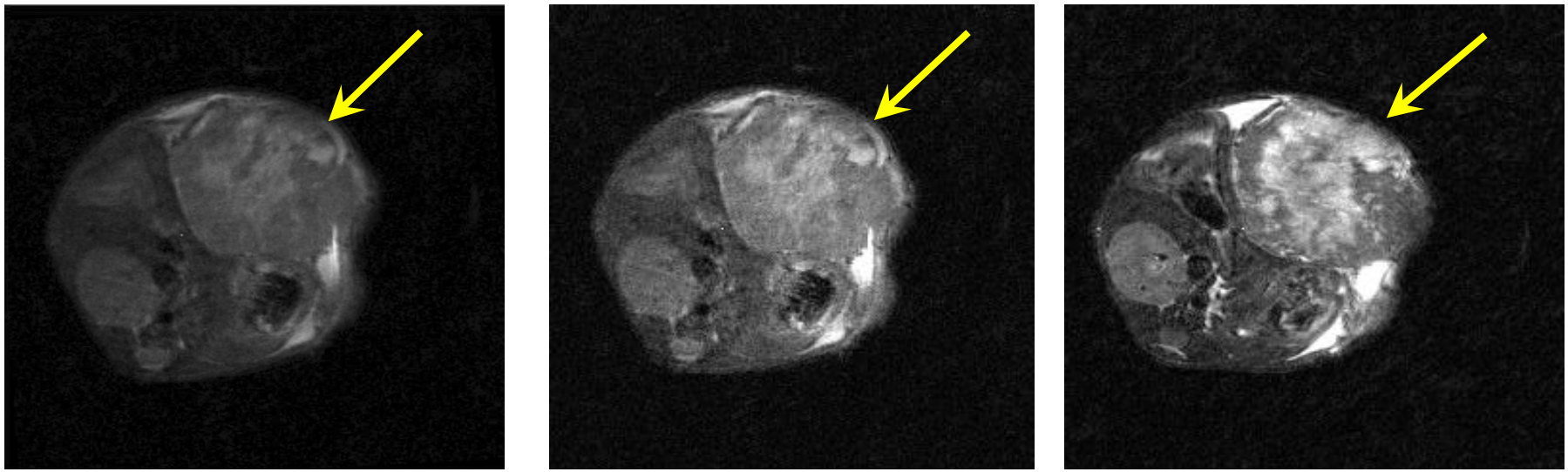
Applications of Nanocapsule Delivery

- Reporter Genes (e.g., Lac-Z; GFP)
- Therapeutic Genes (e.g., P53; RB94)
- siRNAs (e.g., Fl-siRNA; anti-HER2)
- Antisense Oligonucleotides (e.g., anti-HER2)
- Contrast Agents (e.g., Magnevist)
- Small Molecules (several under study)

Systemic Delivery of Imaging Agent (Magnevist) to Tumors *via* Nanocapsules

Enhanced Tumor Imaging *via* Tumor-targeted Nanocapsules

Baseline | **Free Magnevist** | **Nanocapsular Magnevist**



Clinical Trial

- A Phase I clinical trial will commence in 1Q 2006 at the Lombardi Comprehensive Cancer Center
- Safety study in patients with advanced solid tumors
- John Marshall, M.D. will serve as P.I.
- GMP reagents partially produced via NCI's RAID mechanism
- Partially funded by a RO1 Grant from NIDCR for inclusion of oral cancer patients
- Sponsored by SynerGene Therapeutics, Inc.
- Technology development partially funded by a number of STTR/SBIR grants

Conclusions

- **The ligand targeted cationic liposome complex is a nanosized virus-like particle with a condensed DNA core and ligand decorating the surface.**
- **The presence of the ligand bestows exquisite tumor specificity when systemically administered targeting both primary and metastatic disease.**
- **The small size permits efficient and deep tumor penetration.**
- **This “Platform Technology” not restricted to delivering genes.**
- **In addition to genes, this nanocomplex has successfully and efficiently encapsulated and delivered Antisense ODNs, siRNA, Imaging agents and Small molecules.**
- **This approach is now entering a Phase I clinical trial at the Lombardi Comprehensive Cancer Center.**

NanoBioTechnology in Cancer

NBT can be used as a means to achieve the following useful ends:

- **Earlier and/or more accurate diagnoses**
- **More efficacious and/or less toxic treatments**
- **Accelerated development of novel interventions**

Contributors

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