



Polymeric Nanoparticles for Nucleic Acid Delivery

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Disclosures

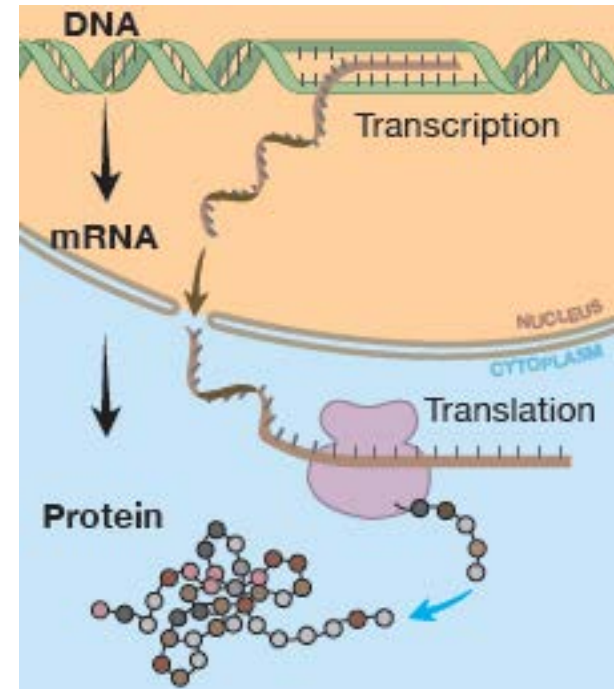


Potential conflicts of interest are managed by the Johns Hopkins
Medical Institutions Committee on Outside Interests

Polymeric Nanoparticles for Intracellular Delivery



- Deliver sensitive biological cargos like proteins, peptides, DNA, & RNA
- Can potentially cure a disease, not just treat symptoms:
 - Genetic Diseases
 - Acquired Diseases



Viral Gene Therapy Tragedies



Gene therapy put on hold as third child develops cancer

Erika Check, Washington

Scientists have halted clinical trials of gene therapy to treat a rare immune disorder — less than a year after the trials were

regulatory authority AFSSPS announced that a child who was treated by Fischer in April 2002 now has cancer.

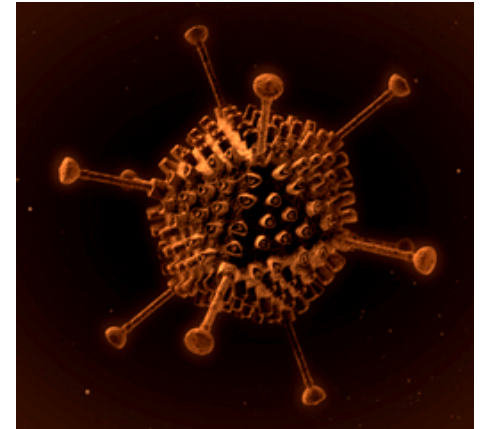
As a result, Fischer's trial and similar ones

Researchers and regulators reflect on first gene therapy death

The Recombinant DNA Advisory Committee (RAC), the National Institute of Health office responsible for oversight of gene therapy, met on December 12 to examine the clinical trial in which Jesse Gelsinger became the first person to die from the experimental technique of gene therapy. The F

Therapy on trial

The death of a participant in a gene therapy trial has thrown the entire field into question—as it did once before in 1999. Can the field survive this second setback? Virginia Hughes investigates.



Leukemia case triggers tighter gene-therapy controls

A hastily convened advisory committee to the US Food and Drug Administration (FDA) ruled last month that gene-therapy trials of the kind that led to a leukemia-like illness in a French patient, and which were halted because of that

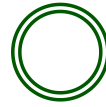
that a case of leukemia occurred in a SCID gene-therapy trial and that the event was related to the treatment.

Although the French patient is the first gene-therapy recipient to develop cancer, scientists have long warned of

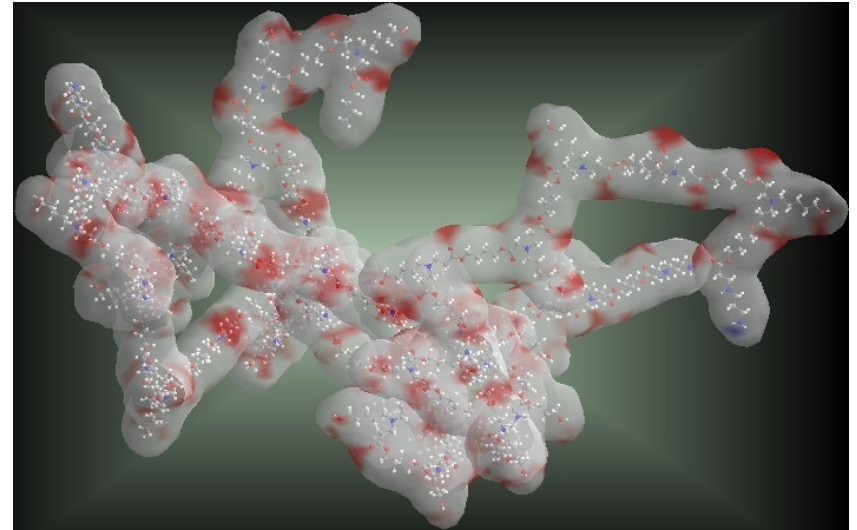
he began a regime of chemotherapy, and the trial was suspended.

Using a new PCR-based technique, Christof Von Kalle of Cincinnati Children's Hospital (and formally at the University of Freiburg Medical

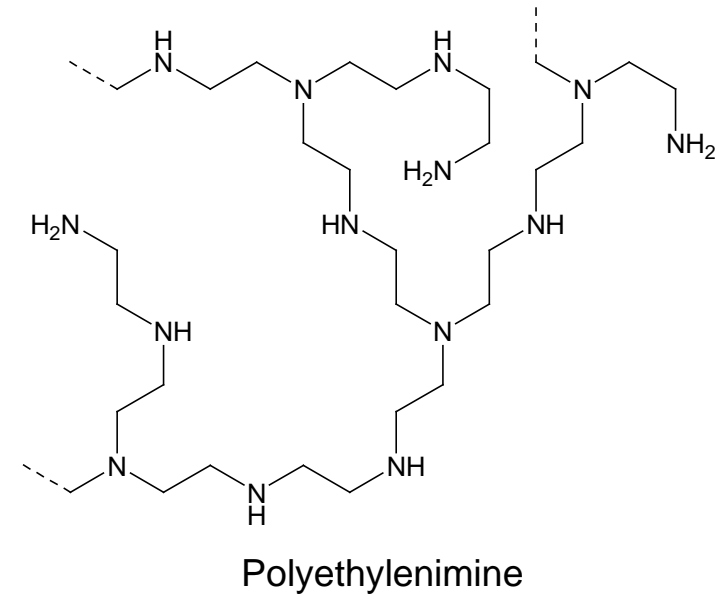
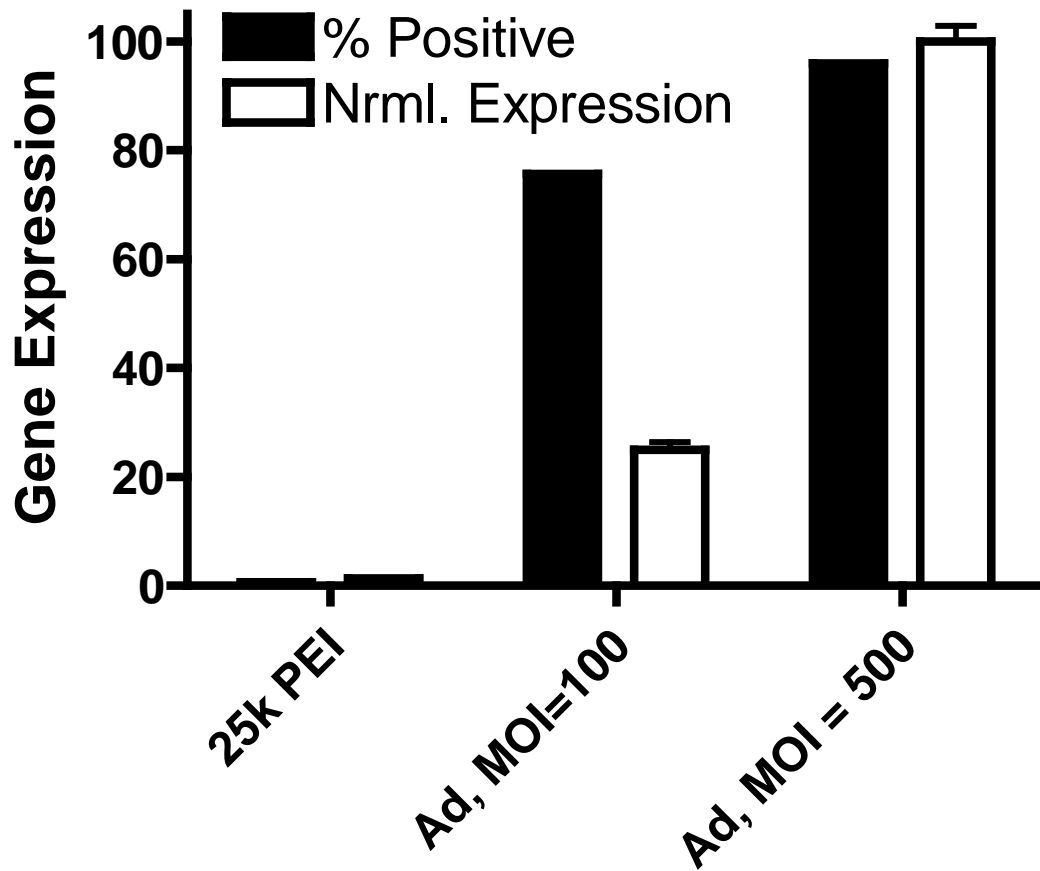
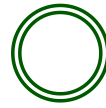
Advantages of Polymers for Gene Therapy



- **Safety**
 - Minimal immune response
 - Minimal cellular toxicity
 - Not carcinogenic
- **Large cargo capacity**
- **Flexibility to target different cells, tissues**
- **Low resistance to repeated administration**
- **Ease of production and quality control**

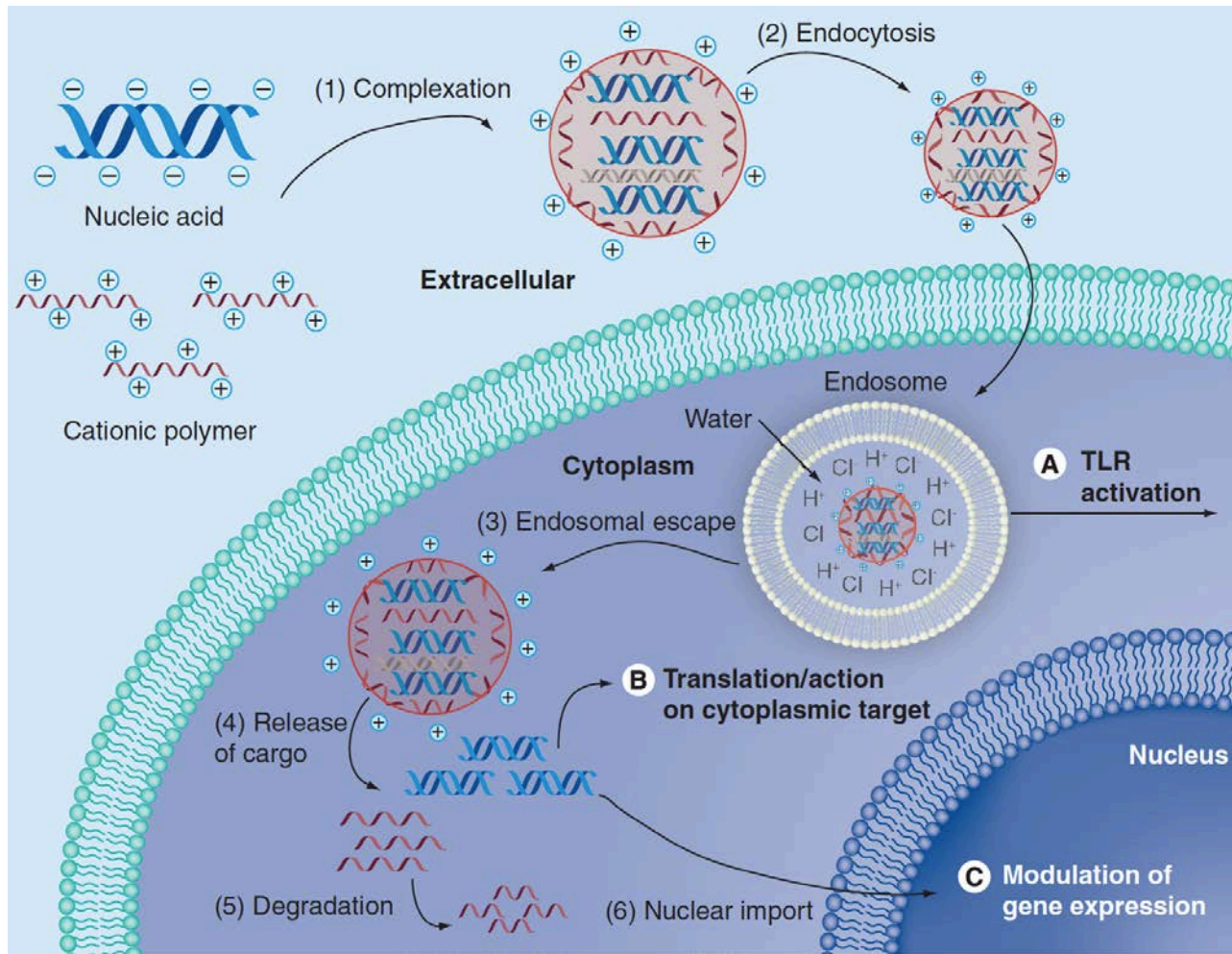


Non-viral Gene Delivery is Ineffective

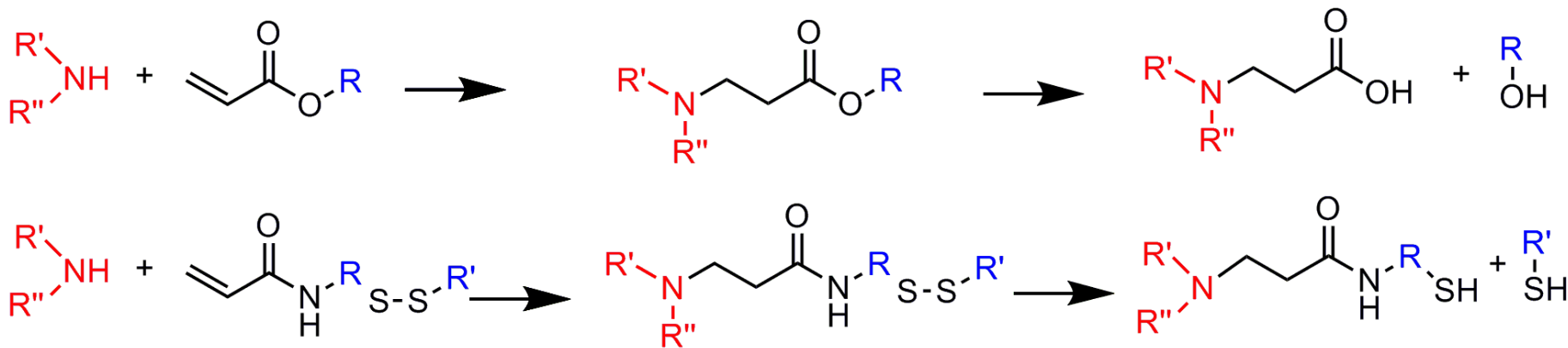


Polyethylenimine (PEI) is
"the gold standard"

Mechanism of Intracellular Delivery

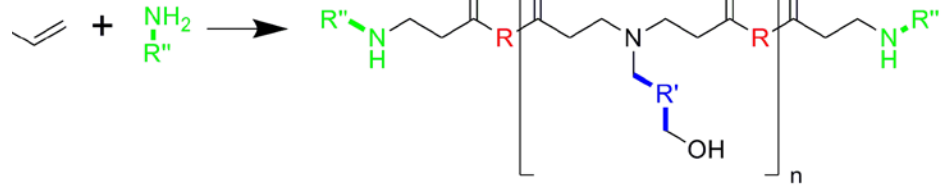
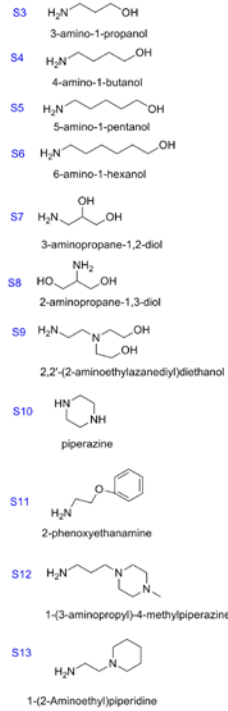
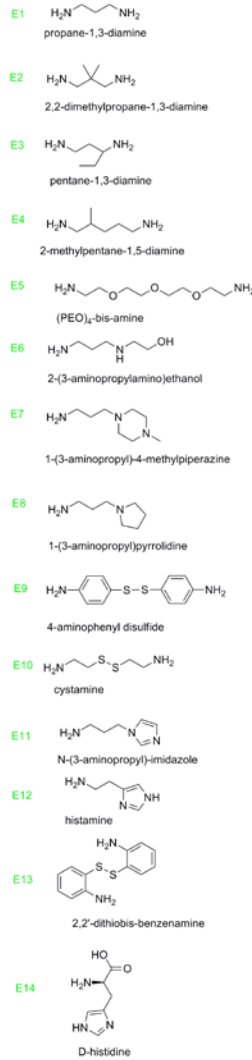
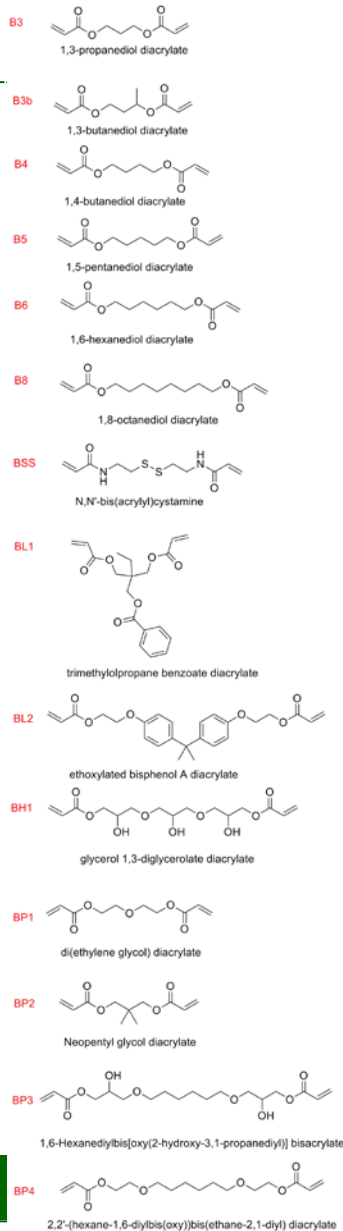


Hydrolytic Poly(Ester Amine)s and Reducible Poly(Amido Amine)s



- One step reaction: amine monomer conjugate addition to diacrylates or diacrylamides
- High-throughput synthesis: Unique polymers, high diversity
- No purification needed: Synthesis neat or in DMSO
- No byproducts, no side reactions
- Tunable polymer function

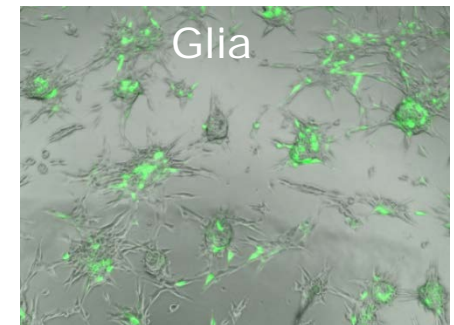
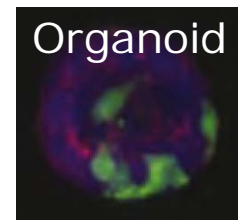
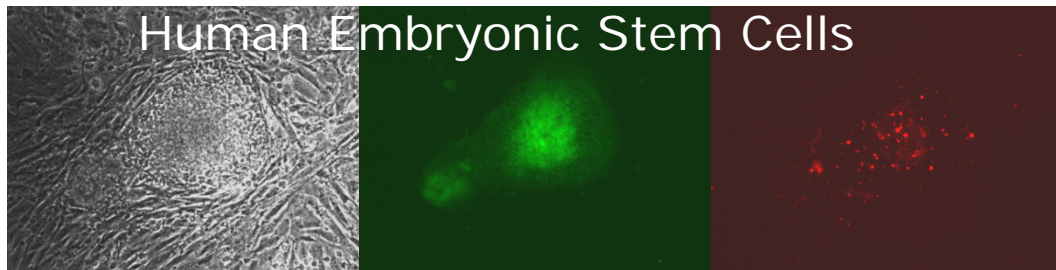
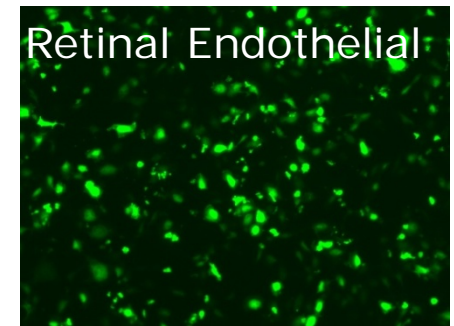
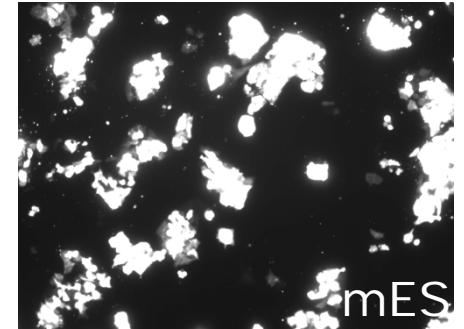
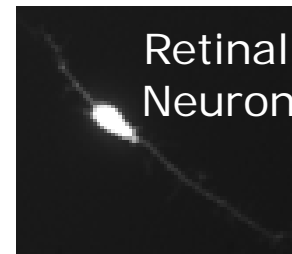
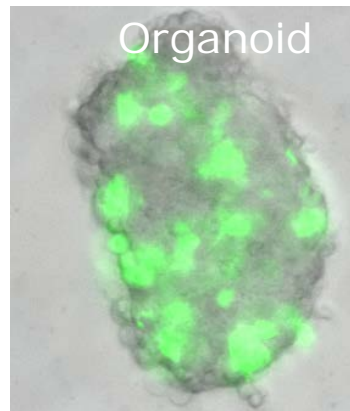
Biomaterial Libraries



- 1,000+ polymers in library and growing
- Structural diversity
- Tune cargo binding and release
- Tune degradability
- Tune cell-material interactions
- Non-cytotoxic

Gene Delivery Nanoparticles

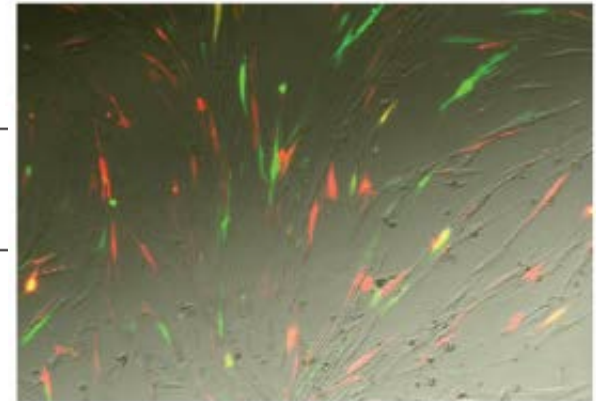
- DNA/RNA Delivery
- Virus-like efficacy
- Non-viral advantages



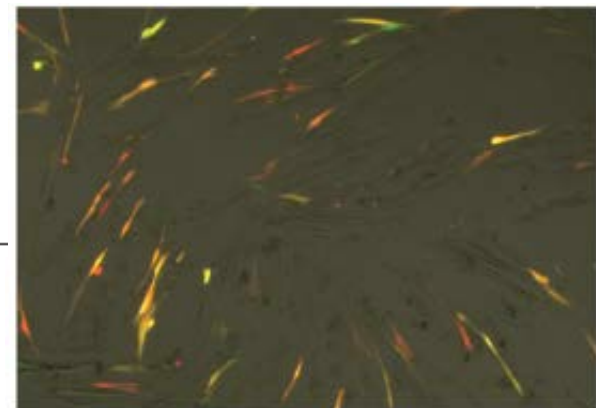
Plasmids per Particle



Polymer	wt/wt	Mode plasmids/particle	Average plasmids/particle
B4S4E7	40	57	120
B4S4E7	60	63	110
B4S5E7	60	29	90
B5S3E7	60	23	45
B5S3E7	100	17	30
B5S5E1	100	23	35
PEI 25 kDa	2	49	90

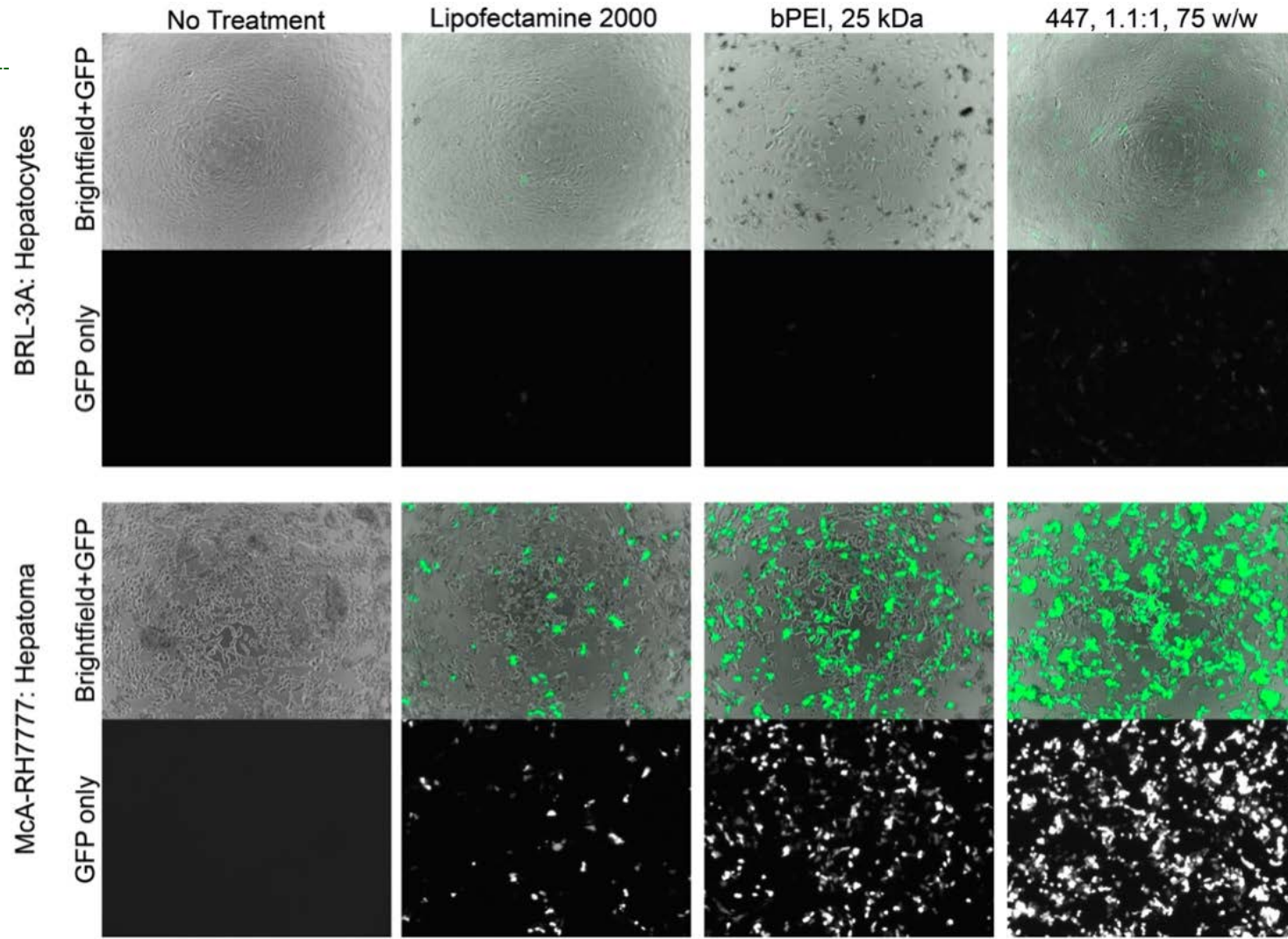


B4S4E7, Day 1 GFP & DsRed in separate particles, same day (Day 3, 12ug DNA)

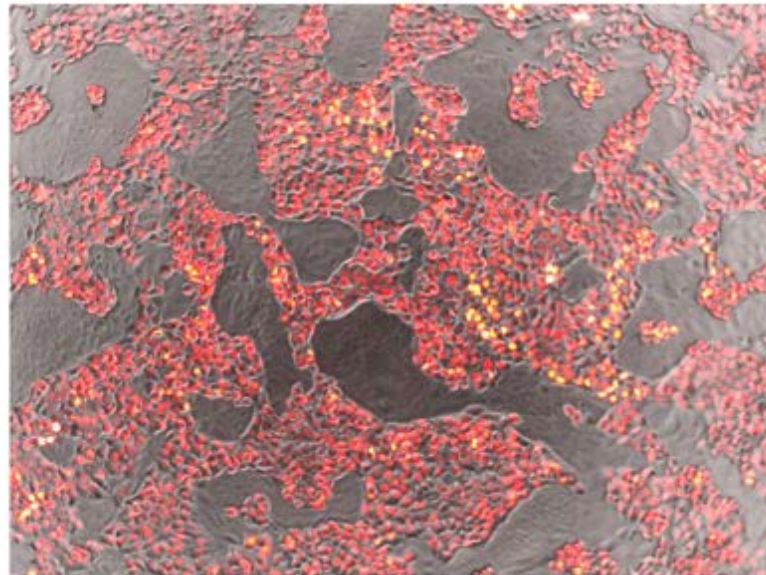
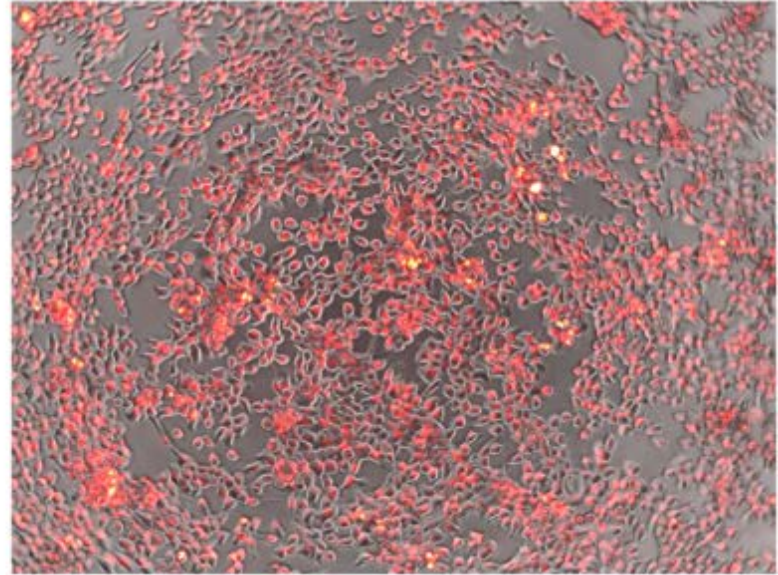
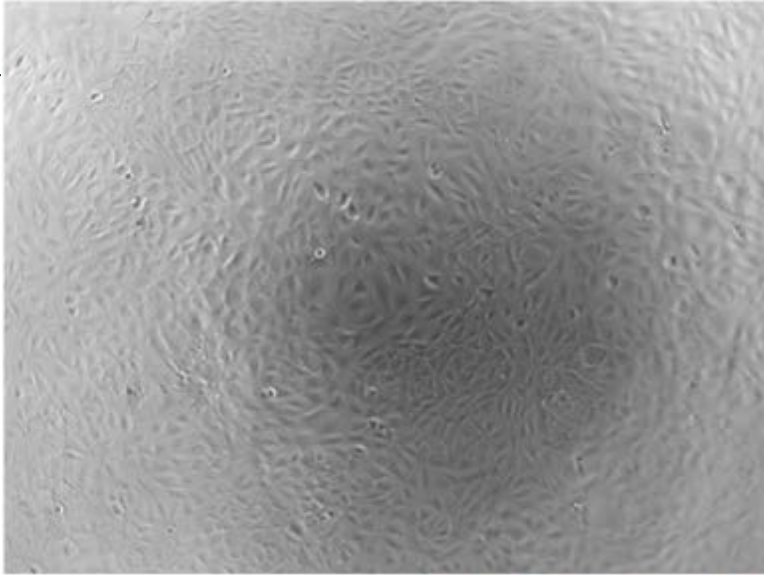


B4S4E7, Day 1 GFP & DsRed together in same particle, same day (Day 3, 6ug DNA)

Cancer vs. Non-cancer: Liver

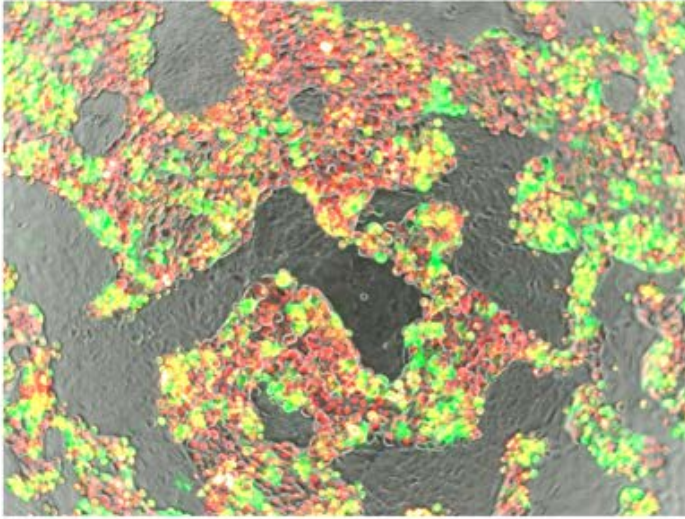


Cancer vs Non-cancer: Liver

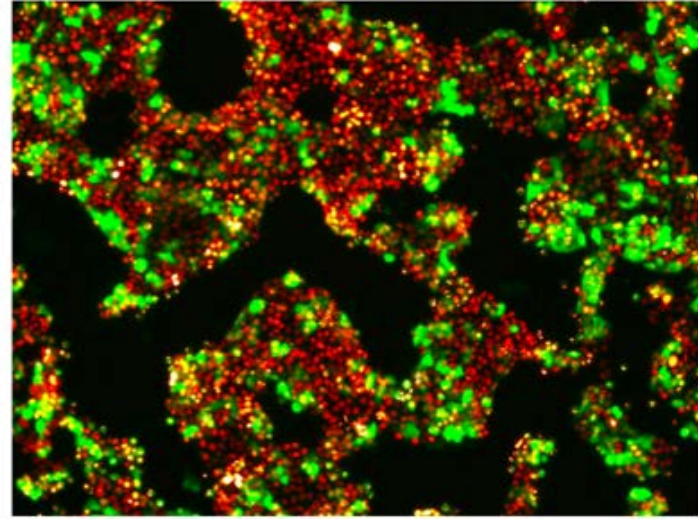


Polymer 457 (50 w/w)

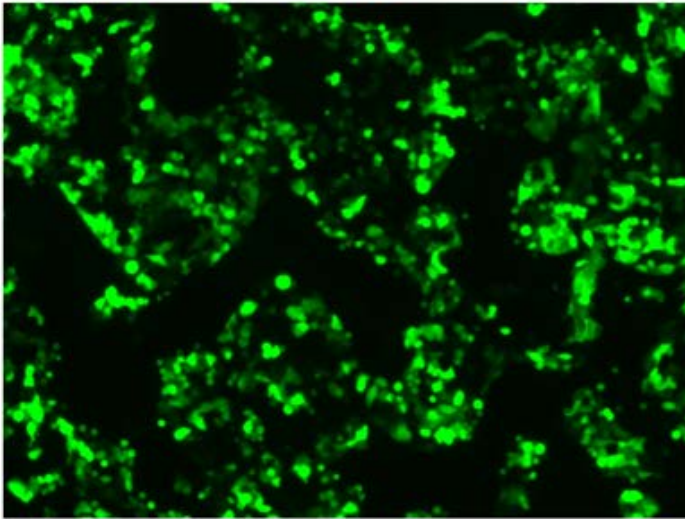
Brightfield+GFP+Cherry



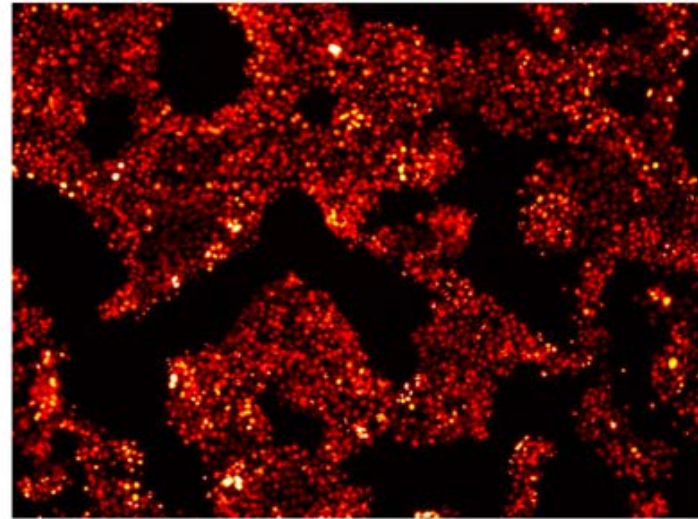
GFP+Cherry



GFP (transfected)

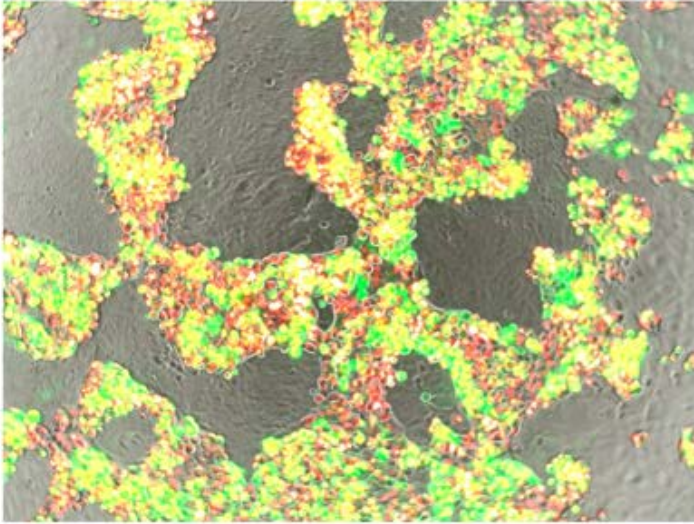


Cherry (hepatoma)

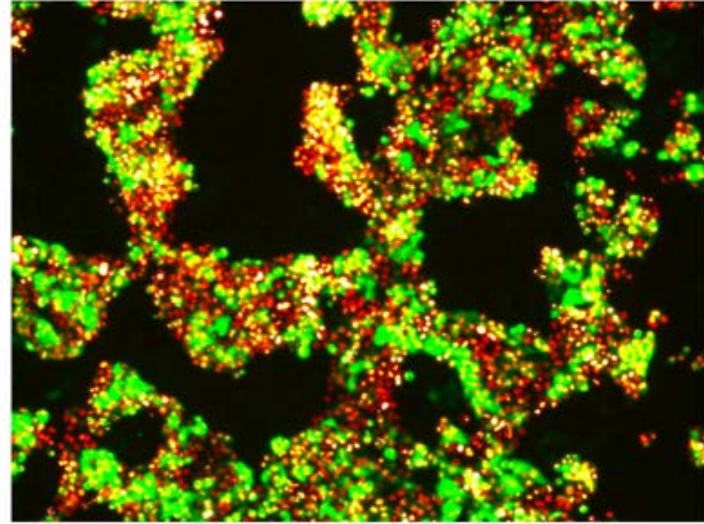


Polymer 457 (75 w/w)

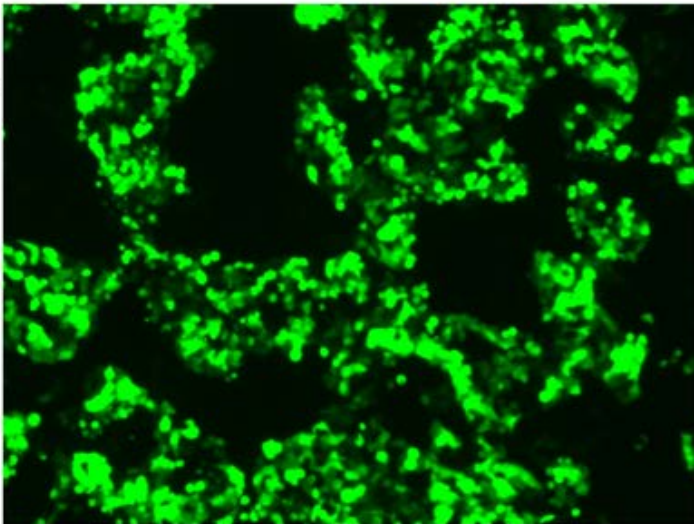
Brightfield+GFP+Cherry



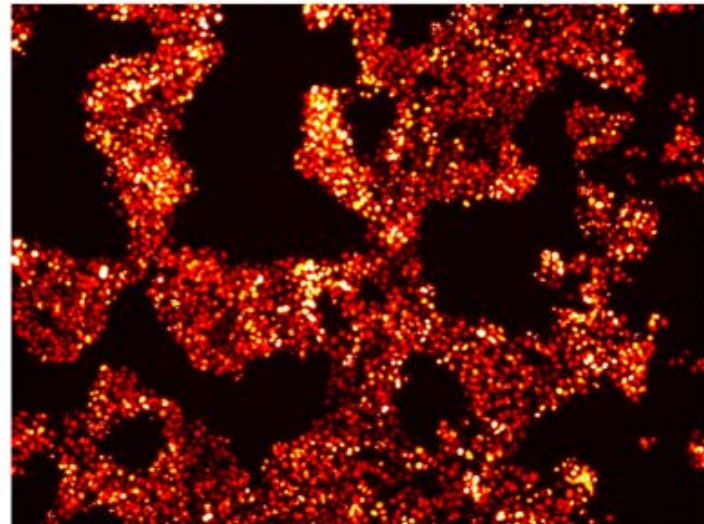
GFP+Cherry



GFP (transfected)

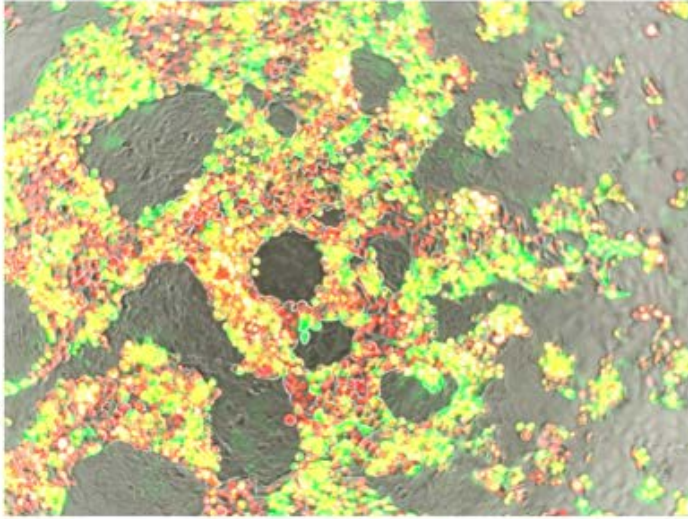


Cherry (hepatoma)

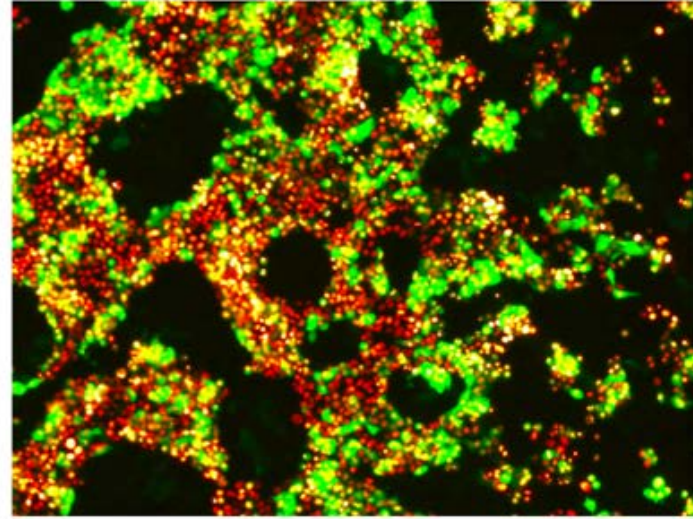


Polymer 537 (w/w)

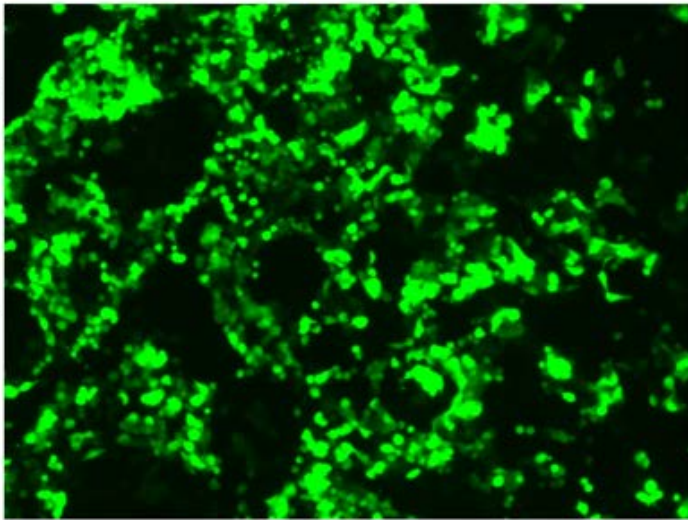
Brightfield+GFP+Cherry



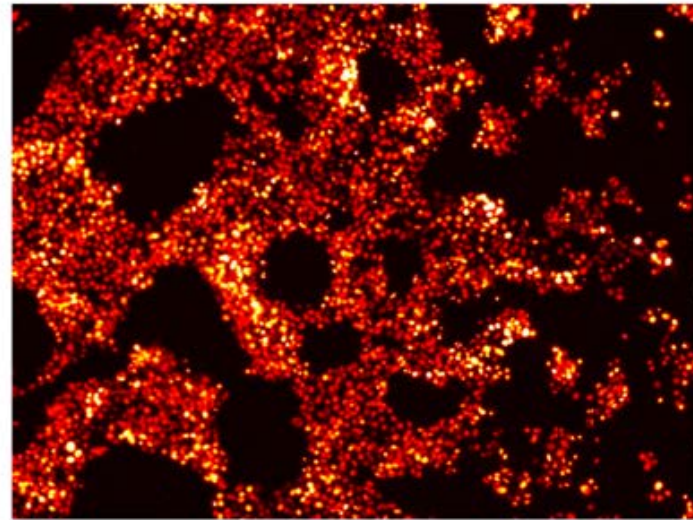
GFP+Cherry



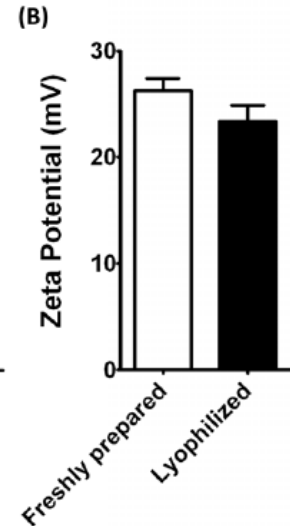
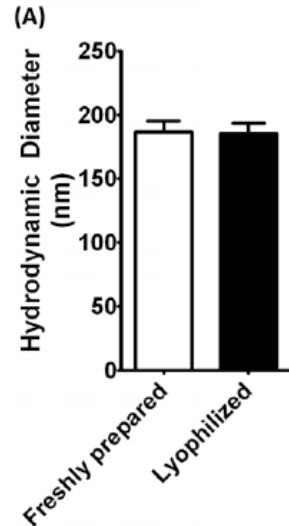
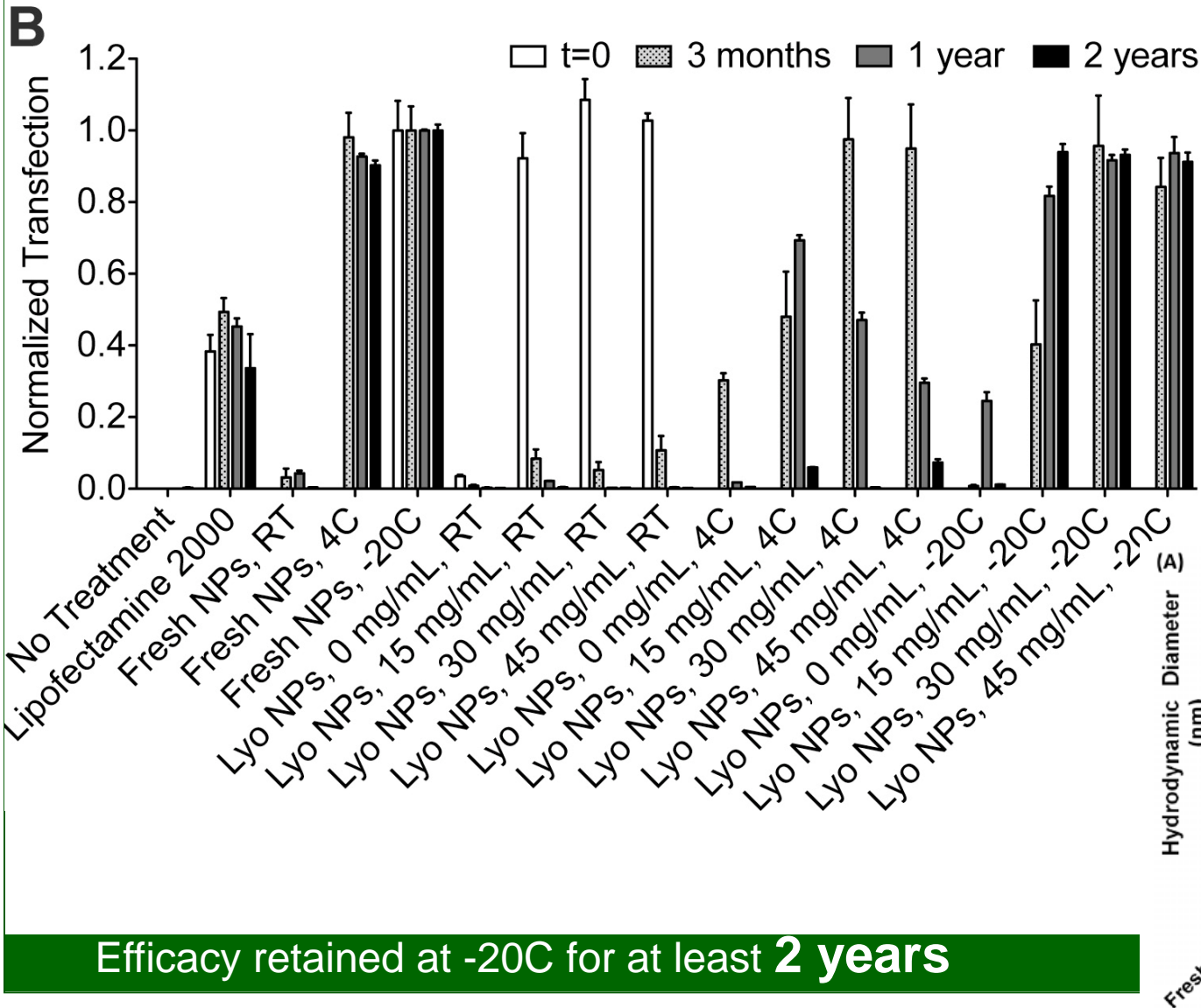
GFP (transfected)



Cherry (hepatoma)

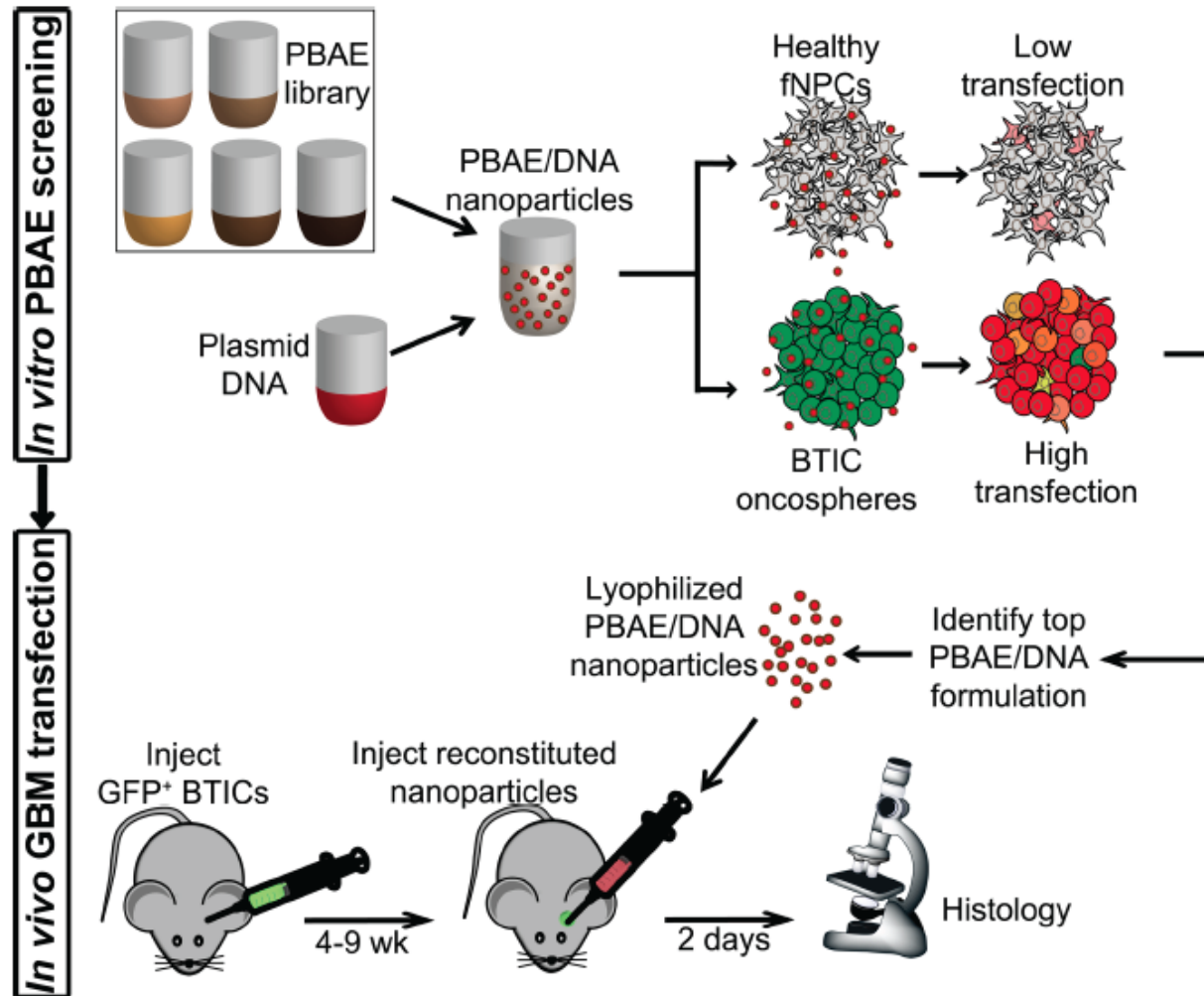


Lyophilized Nanoparticles are Stable

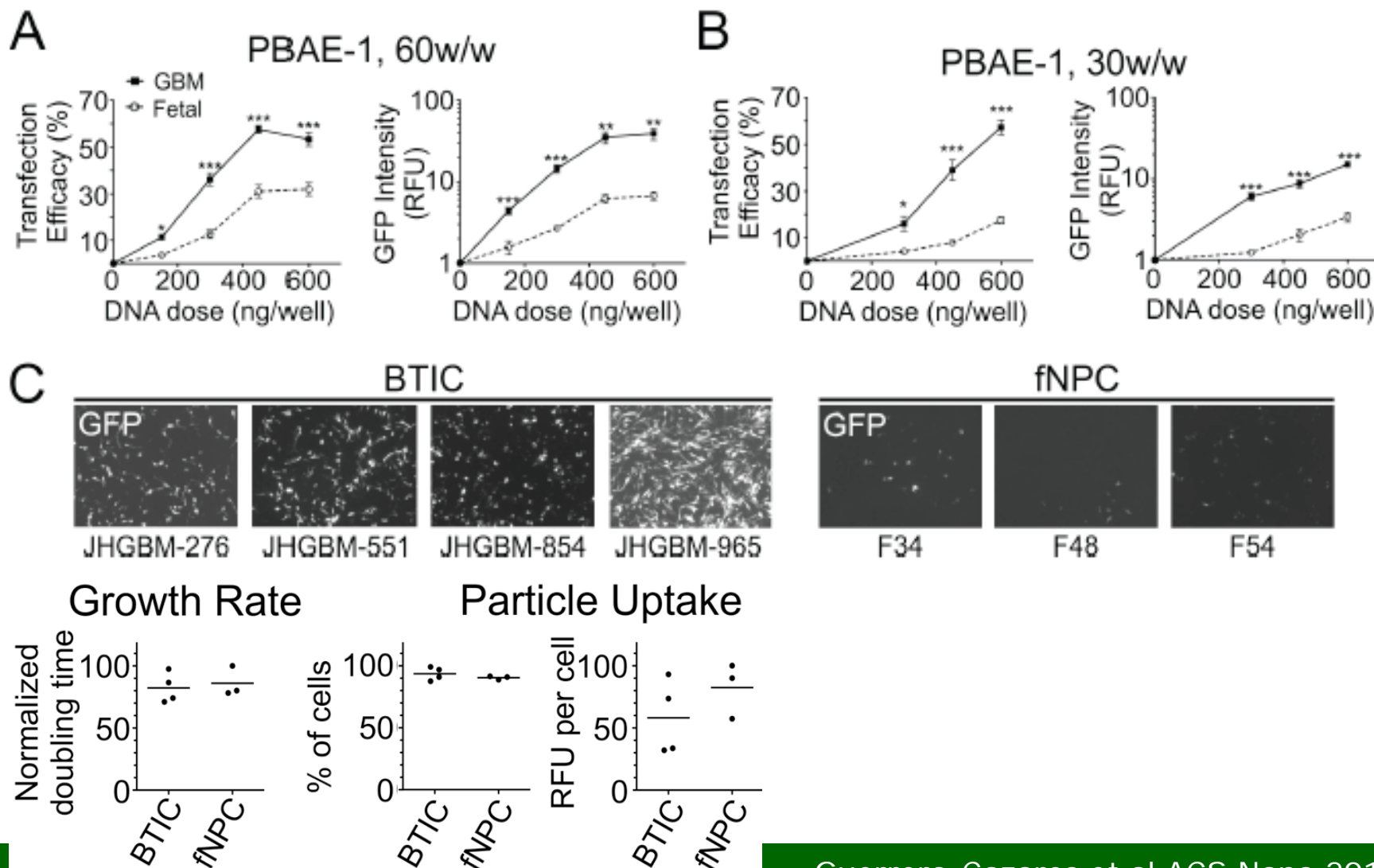


Efficacy retained at -20C for at least **2 years**

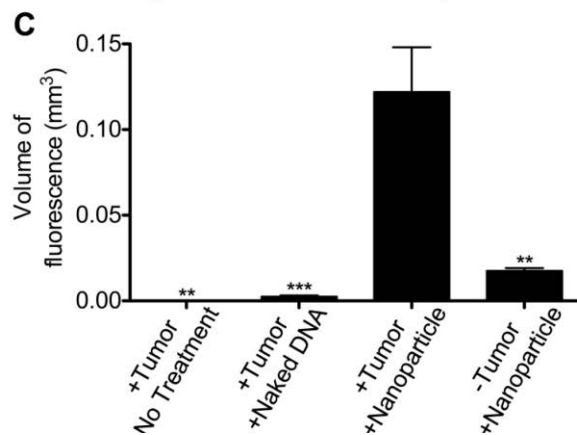
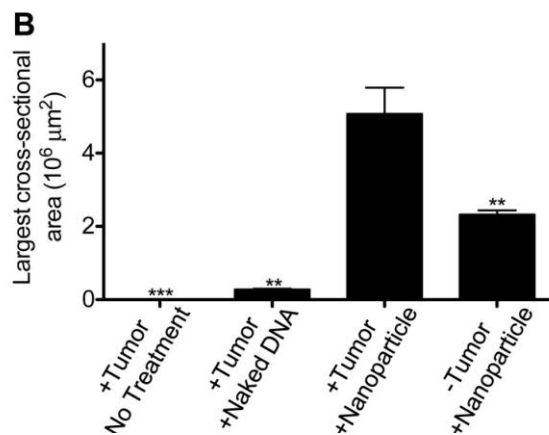
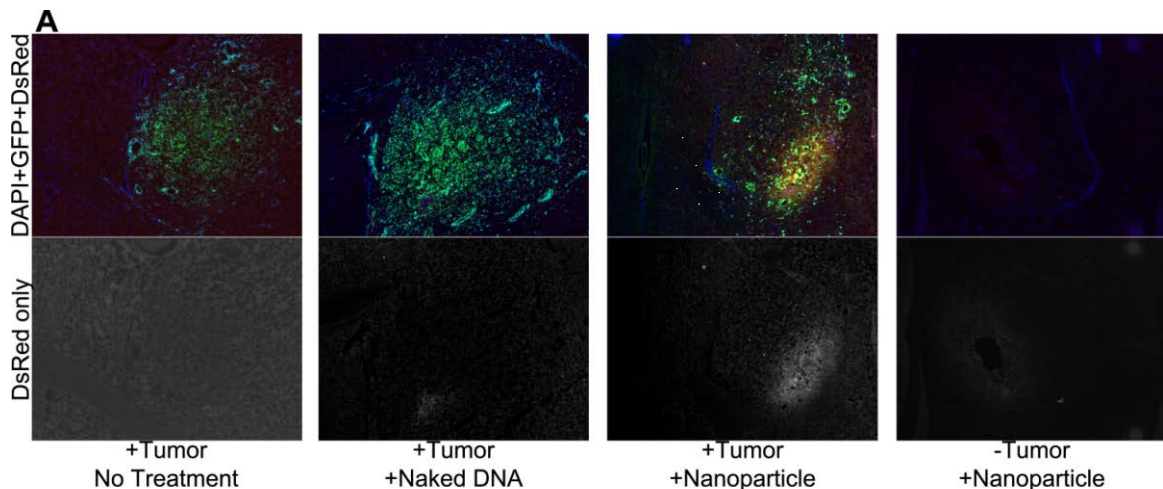
Brain Cancer Strategy



Specificity to Brain Tumor Initiating Cells

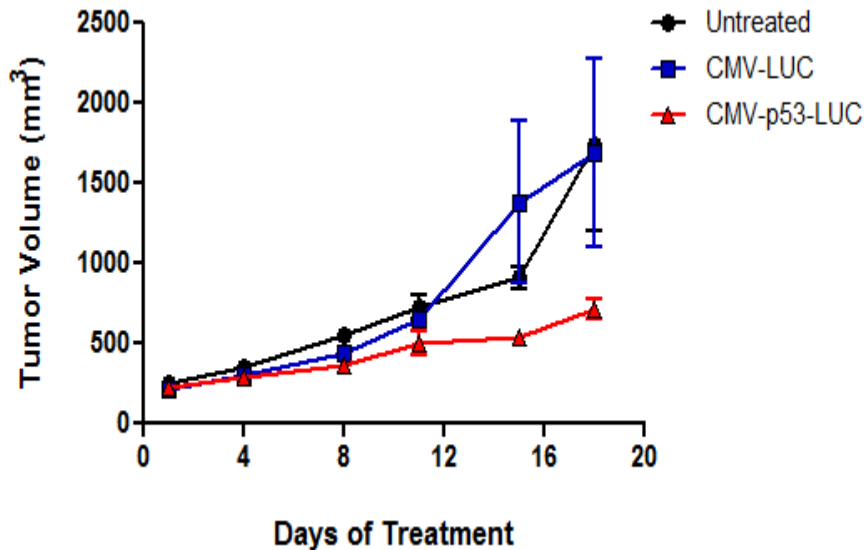
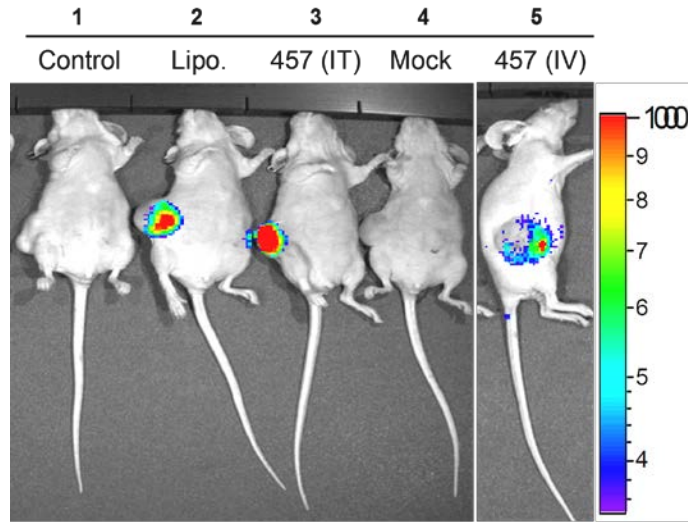


Application to Brain Cancer

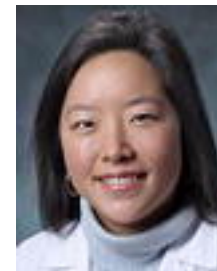


- Patient derived glioblastoma (GB)
- High transfection of 3D GB neurospheres matches 2D monolayer
- Athymic mice intracranially injected with human GB
- 9 weeks later injected with NPs
- Lyophilized particles maintain efficacy for >2 years

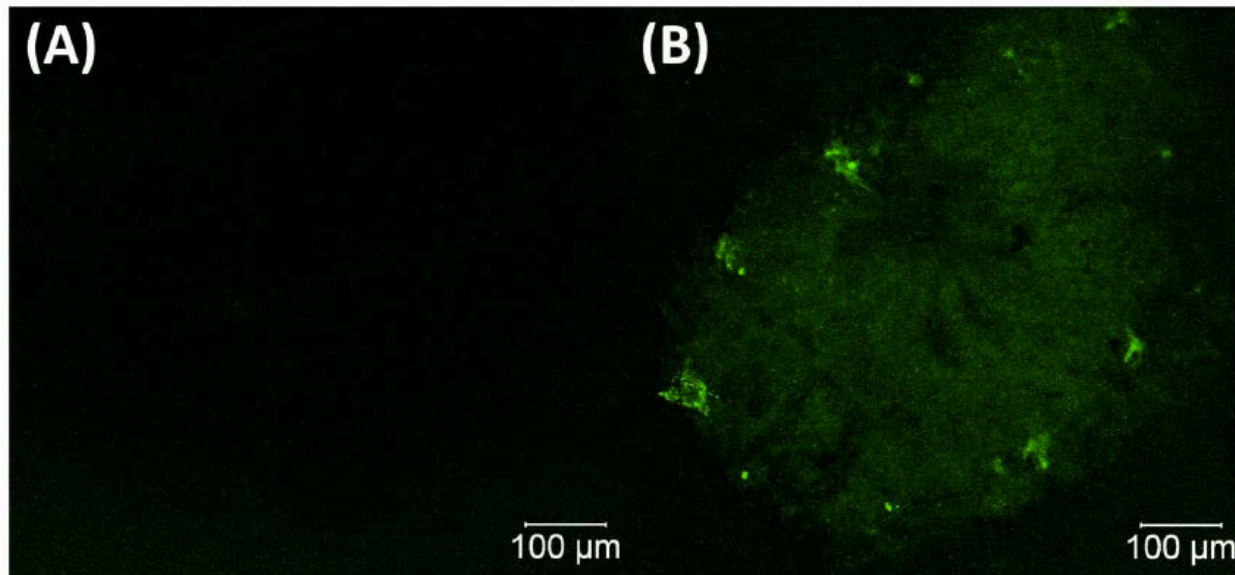
Application to Lung Cancer



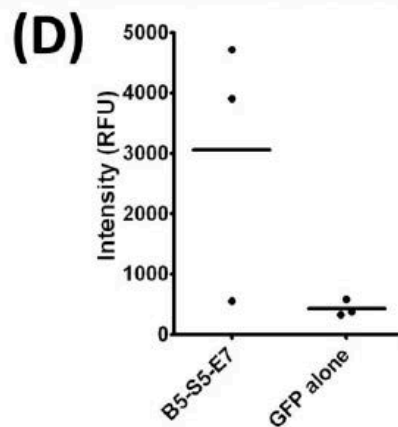
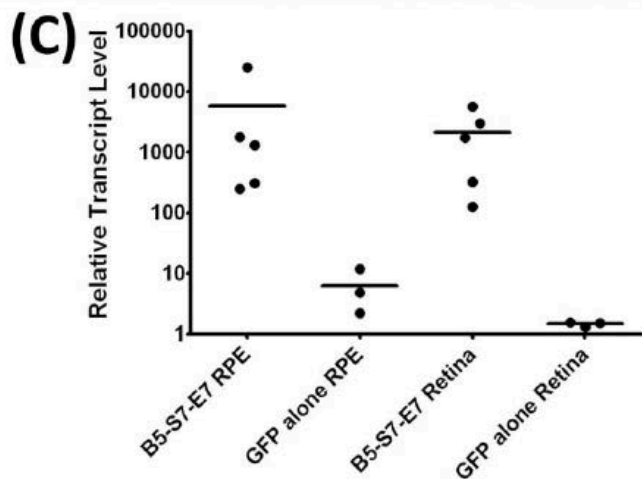
- Small Cell Lung Cancer Model (subcutaneous H446 xenograft)
- TP53 often mutated
- Exogenous expression of TP53 caused apoptosis and reduced tumor growth



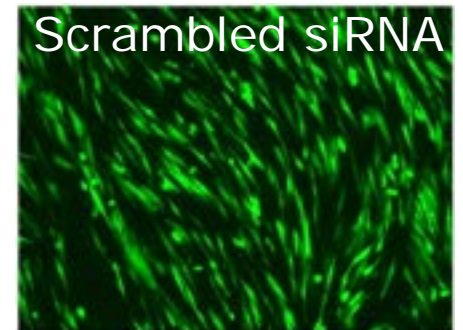
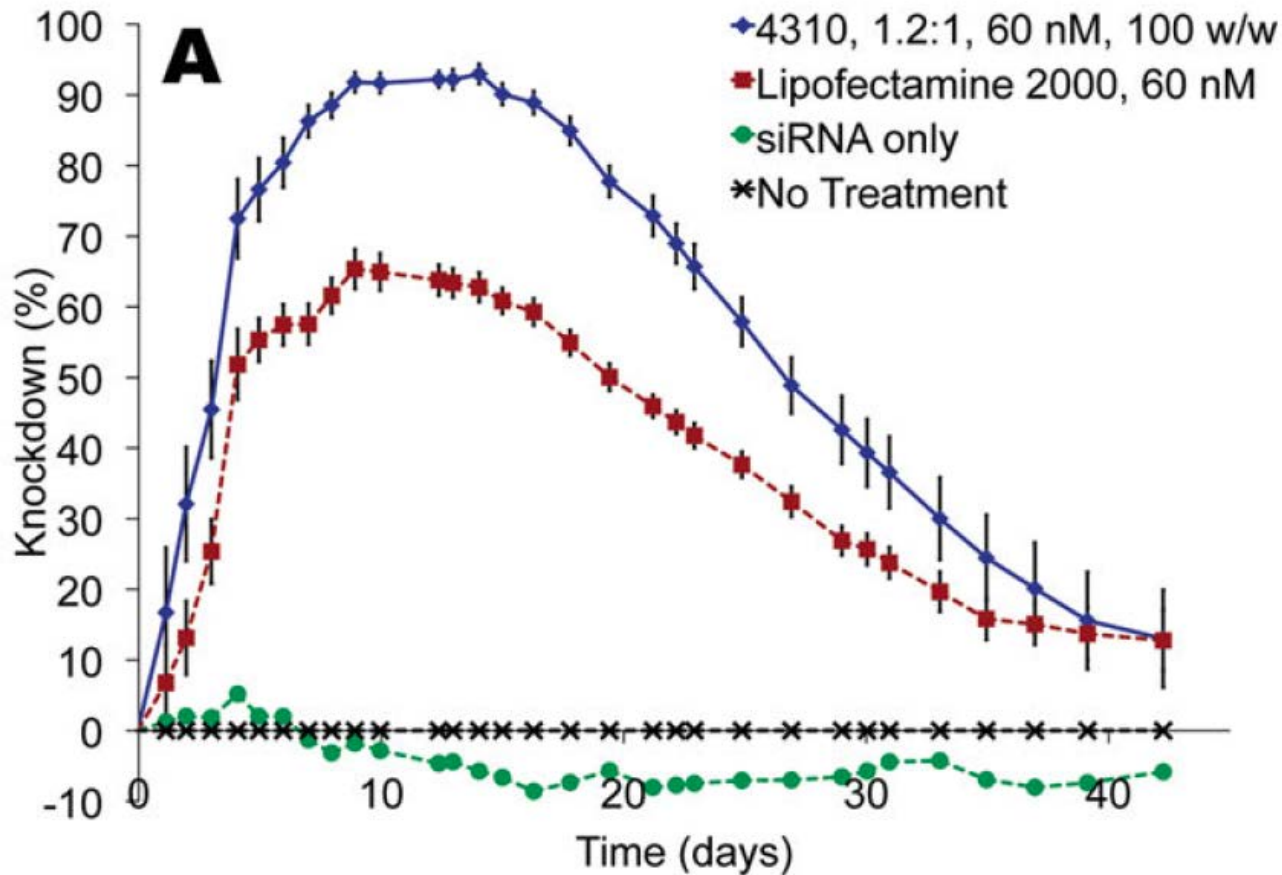
Application to Ophthalmology



- Subretinal Injection
- 1 μg / 1 μL
- Choroid flatmount
72 hrs injection
- mRNA
- Protein
Fluorescence
- Non-cytotoxic

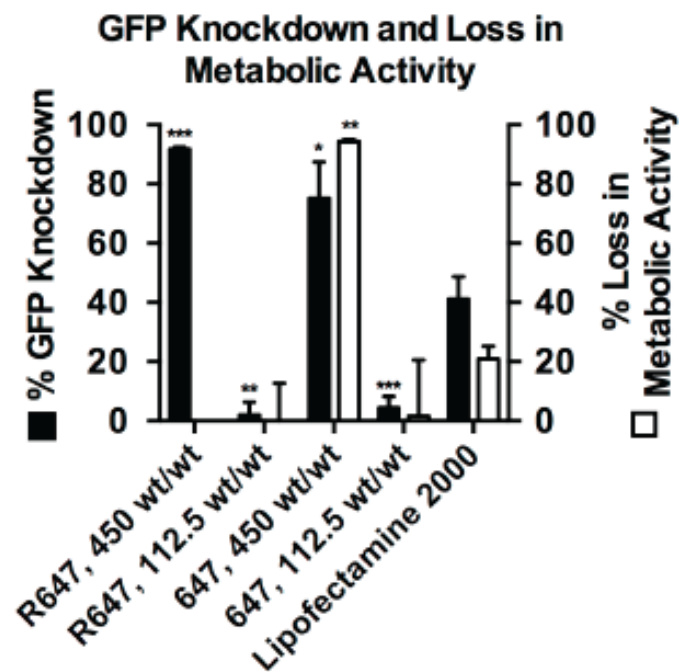
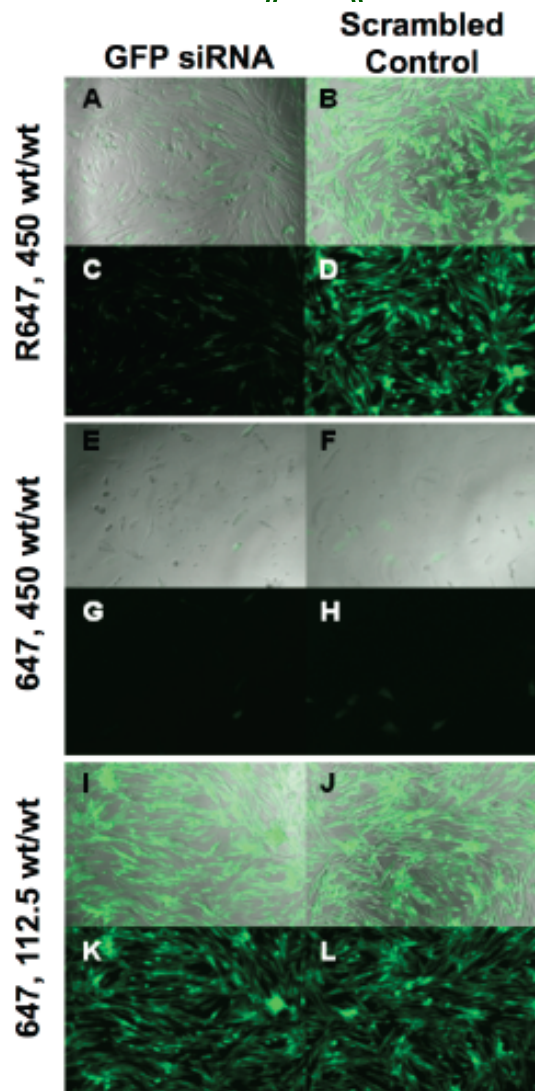
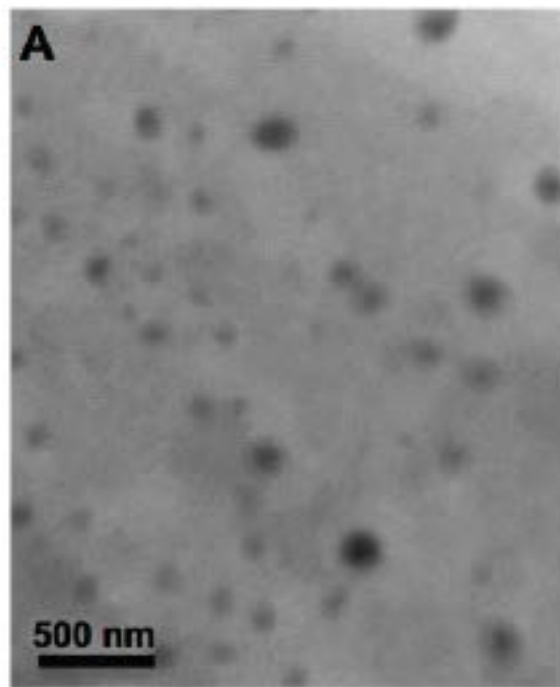


siRNA Delivery Nanoparticles



Knockdown
after 12 days
(4310)

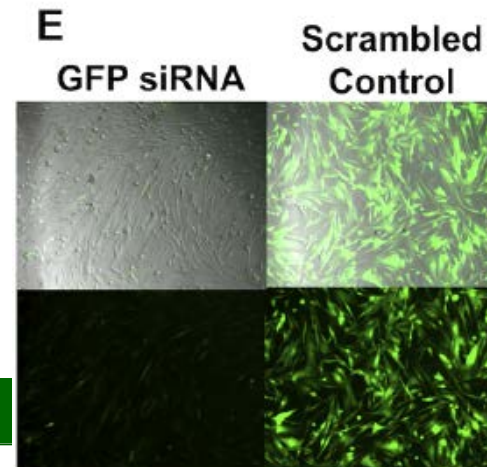
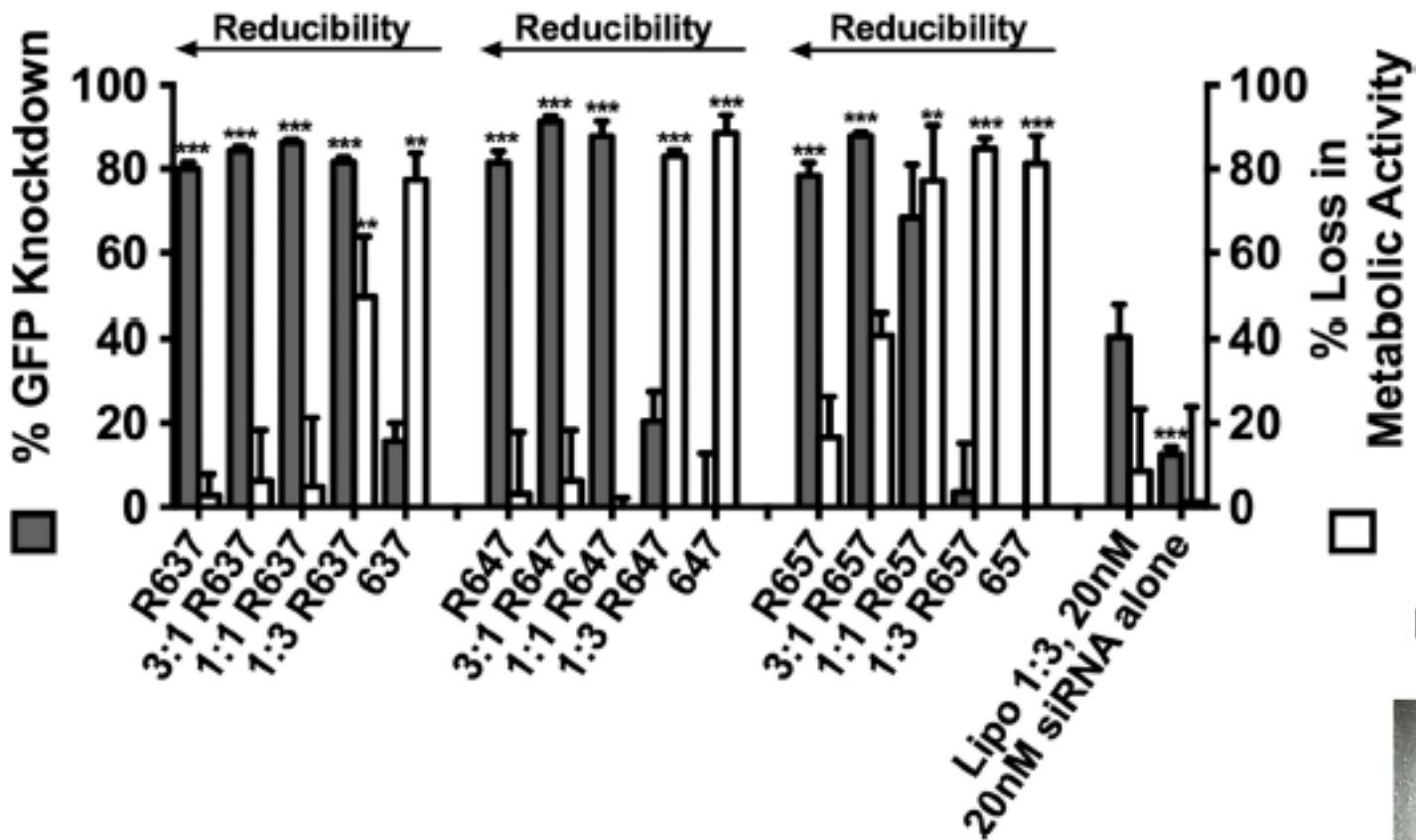
Bioreducibility Reduces Cytotoxicity and Improves Knockdown



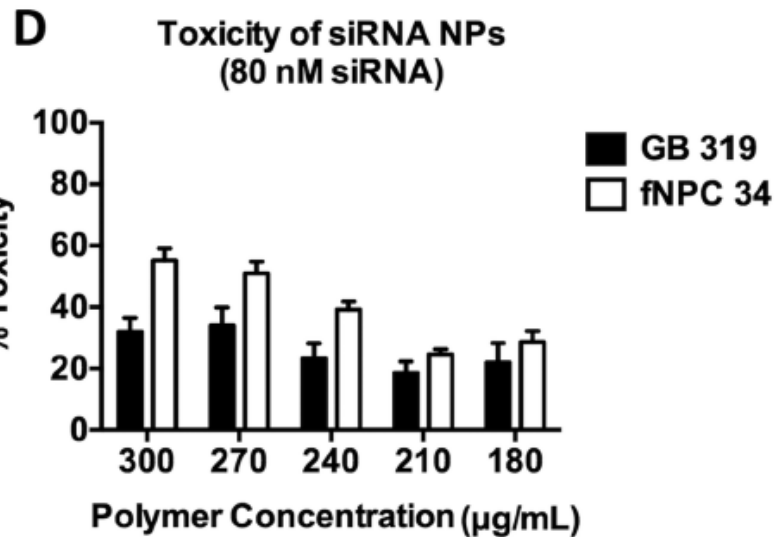
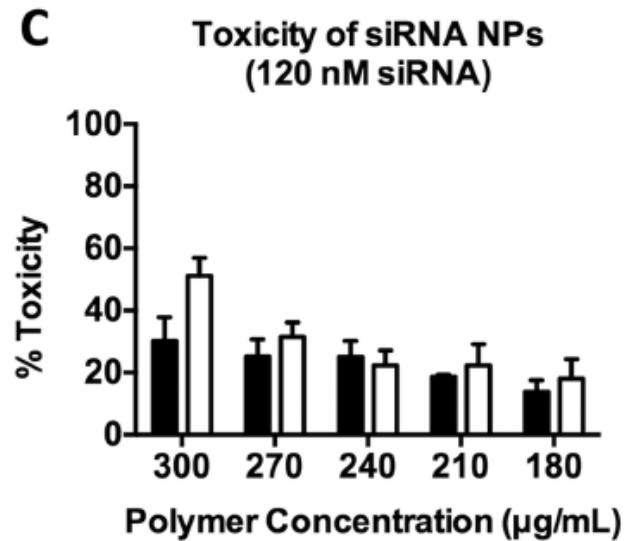
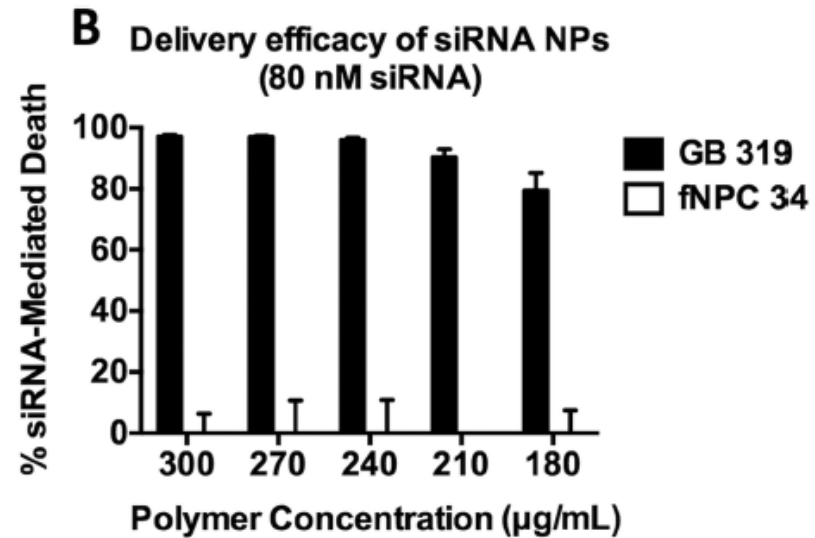
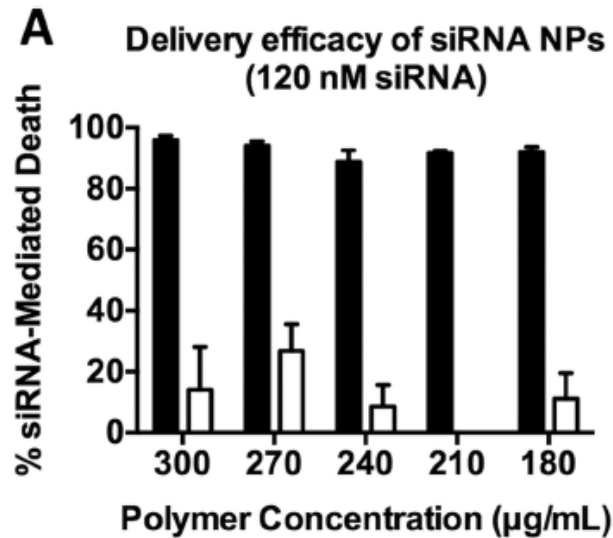
Intermediate Bioreducibility is Optimal



GFP Knockdown and Loss in Metabolic Activity



Cancer-specific Killing via siRNA Delivery

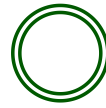


Conclusions



- A polymer library approach allows for enabling technologies such as intracellular delivery of DNA and siRNA
- Applications for Cancer, Regenerative Medicine, Ophthalmology, and Immunology

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