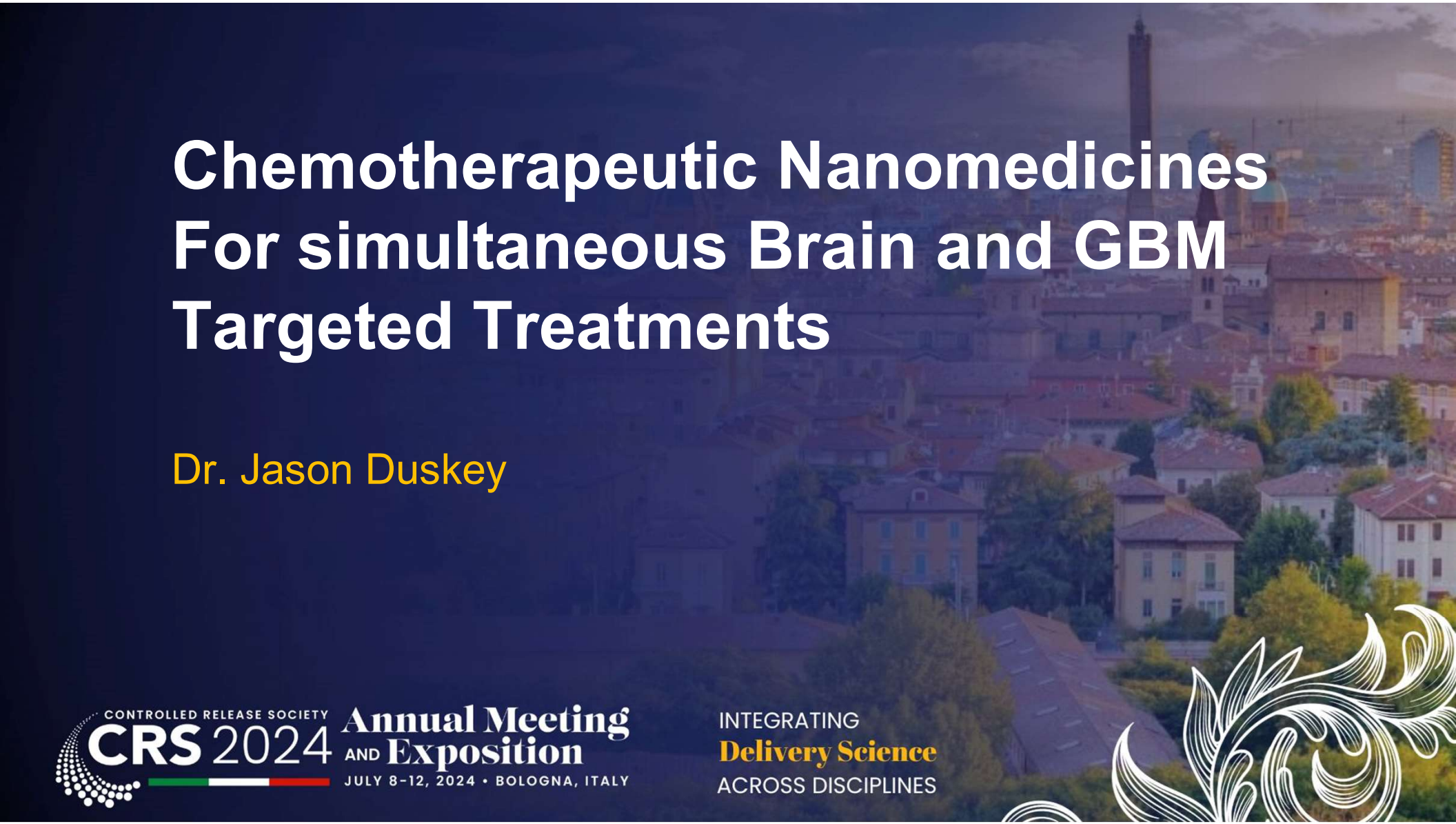
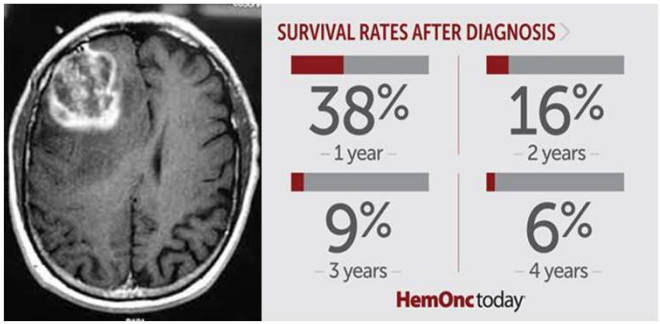
Chemotherapeutic Nanomedicines For simultaneous Brain and GBM Targeted Treatments

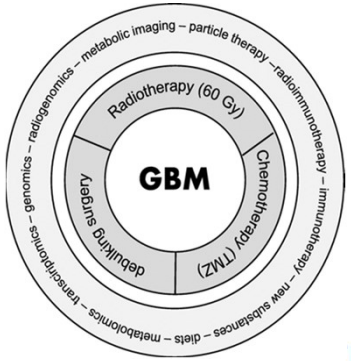
Dr. Jason Duskey

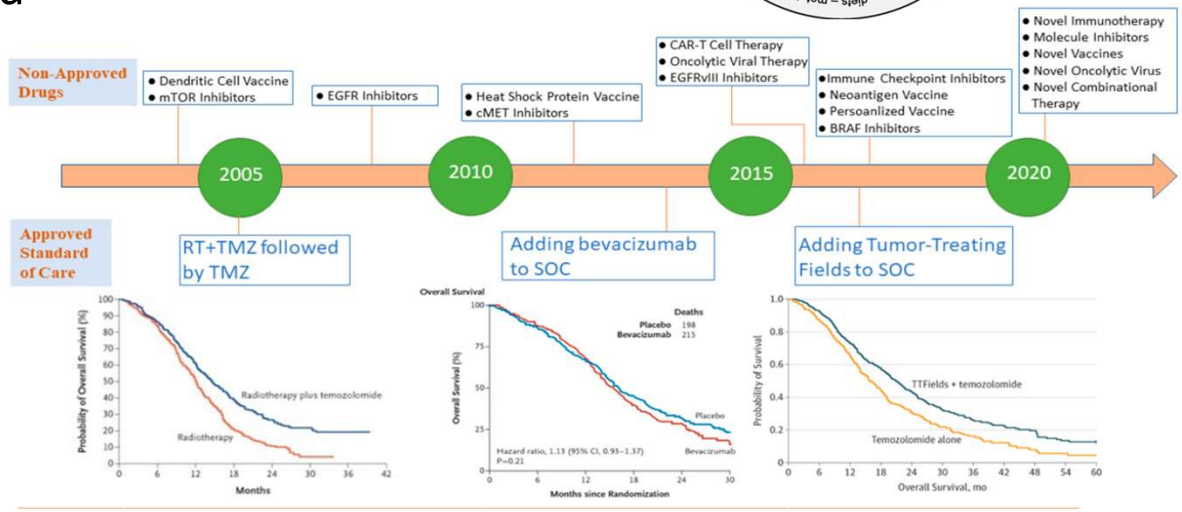


Glioblastoma Multiforme

- Most malignant Brain Tumor
- 17,000 cases a year, 5 in 1000 affected
- Poor Life expectancy
  - Age Linked

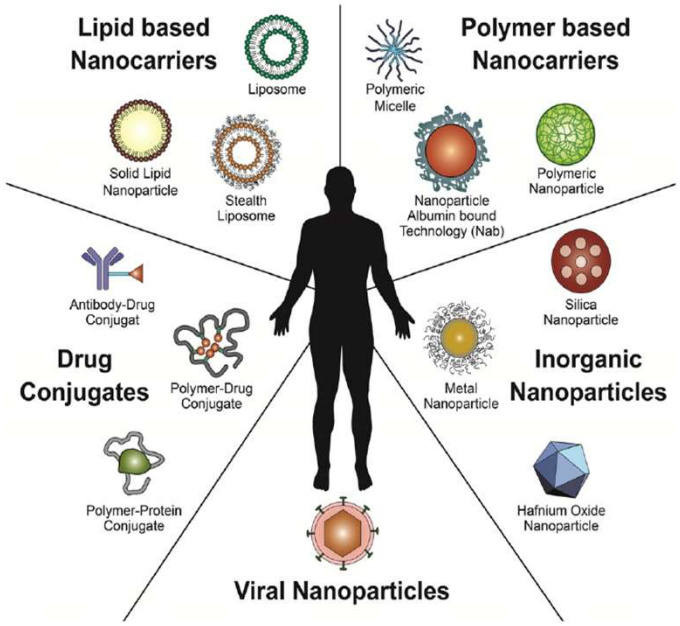


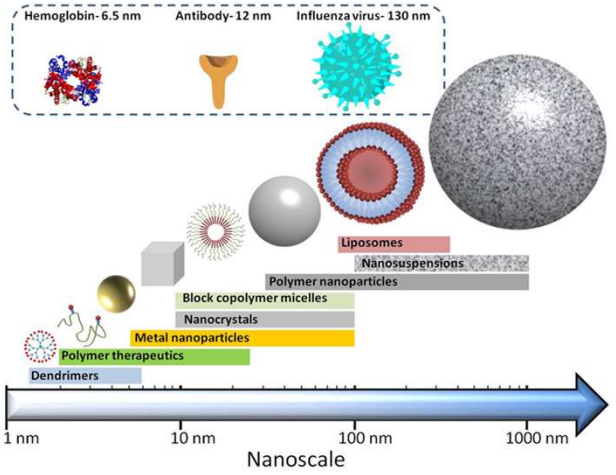


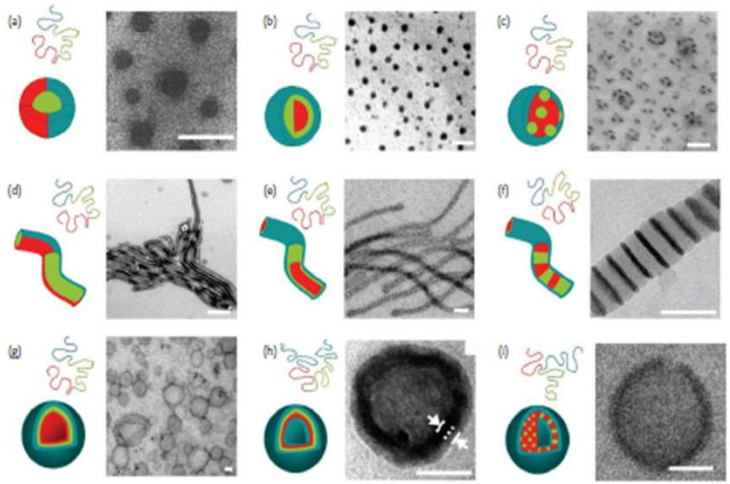


https://cmedresearch.com/spotlights/glioblastoma-experience/ : 10.1093/neuonc/nov029. Epub 2015 Mar 10: https://doi.org/10.3390/ph13110389:
10.1093/neuonc/nov029. Epub 2015 Mar 10: cancer.org
https://www.healio.com/news/hematology-oncology/20190703/despite-incremental-progress-longterm-survival-rates-have-not-budged-in-glioblastoma:
emedicine.medscape.com

Why Nanomedicine?





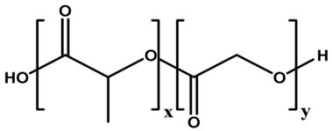


Pharmaceuticals

- Poor Solubility
- Poor Stability
- Poor Bioavailability
- Toxicity
- Untargeted - (Off Target Effects)

PLGA nanoparticles

PLGA: poly(lactic-co-glycolic acid)



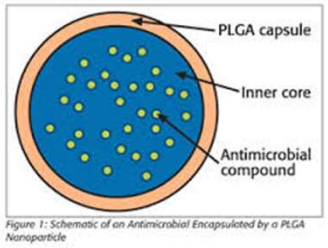
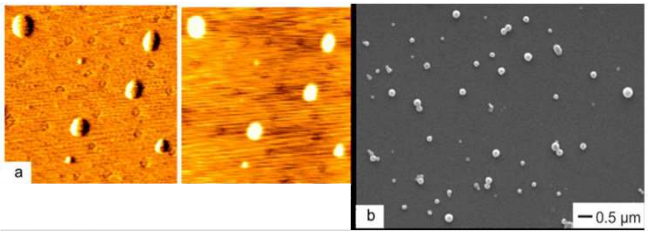


Figure 1: Schematic of an Antimicrobial Encapsulated by a PLGA Nanoparticle

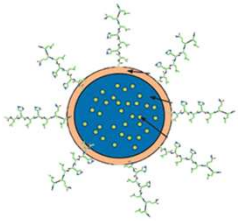
- Safe (FDA approved)
- Not extremely expensive
- Possible to chemically modify with ligands
- Double emulsion nanoparticles
  - 200 nm
  - -20 mv charge
  - Possible to entrap various molecules

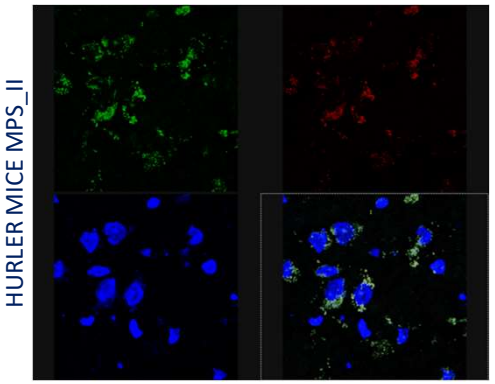


Nanomedicine (NPs+PROTEIN): ACCUMULATION in NEURONS

Targeted PLGA (Pre-modified)

- G7 peptide
  - Targets Brain
  - Opioids receptor derivative
  - Can be linked to PLGA
  - Still forms similar nanoparticles
- Can carry small molecules, peptides, proteins, and enzymes to the brain
- 10% of injected dose





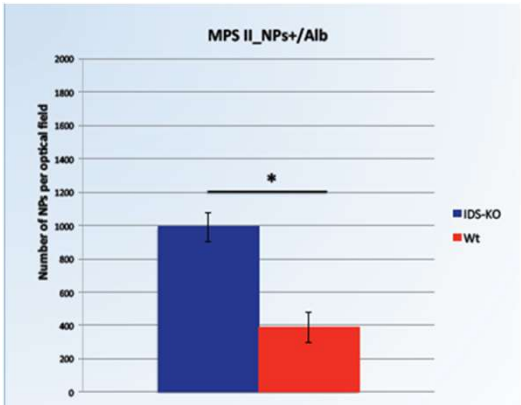
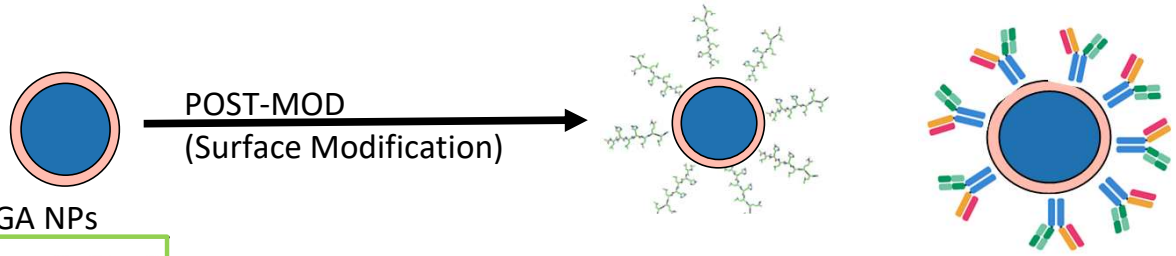


Fig.5: Number of NPs per optical field evaluated on brain slices by confocal microscopy. Hunter and wild-type mice (both *n=3*) were injected with NPs+/Alb.
** p<0.05*

doi.org/10.1371/journal.pone.0156452

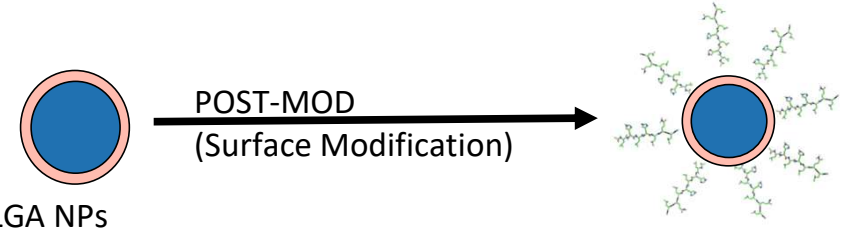
GBM Targeted Nmeds: Formulation Characterization



PLGA NPs

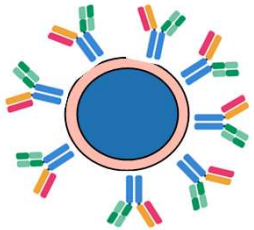
NMed formulation	Ligand Amount (ug)
Optimized NMed	0
NMeds with mock reaction	0
g7-NMeds	1
PAAVF-NMeds	
M08-NMeds	
M08J-NMeds	
g7-NMeds	10
PAAVF-NMeds	
M08-NMeds	
M08J-NMeds	

GBM Targeted Nmeds: Formulation Characterization

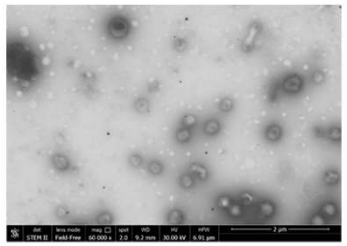


PLGA NPs

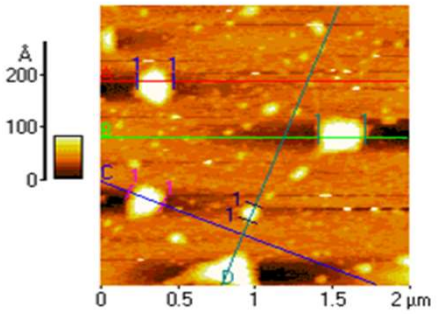
NMed formulation	Ligand Amount (ug)	Size (nm)	PDI	Z potential (mV)	% Residual of surfactant	Weight yield %
Optimized NMed	0	157 ± 8	0.07 ± 0.01	-45.6 ± 4	12 ± 5	87 ± 9
NMeds with mock reaction	0	166 ± 10	0.12 ± 0.01	-33 ± 10	10 ± 7	93 ± 6
g7-NMeds	1	155 ± 13	0.09 ± 0.03	-34 ± 11	9 ± 8	92 ± 5
PAAVF-NMeds		156 ± 9	0.08 ± 0.01	-25 ± 9	10 ± 5	112 ± 10
M08-NMeds		164 ± 10	0.16 ± 0.01	-26 ± 10	11 ± 6	97 ± 9
M08J-NMeds		161 ± 11	0.11 ± 0.02	-31 ± 8	11 ± 5	102 ± 7
g7-NMeds	10	159 ± 10	0.08 ± 0.01	-31 ± 13	8 ± 9	104 ± 5
PAAVF-NMeds		156 ± 12	0.09 ± 0.02	-29 ± 8	10 ± 5	96 ± 3
M08-NMeds		160 ± 14	0.18 ± 0.03	-32 ± 9	11 ± 6	119 ± 4
M08J-NMeds		159 ± 11	0.10 ± 0.02	-29 ± 11	9 ± 9	110 ± 4



- Size – 150 - 170 nm
- Monodisperse (pdi < 0.2)
- WY > 90%
- Consistent surfactant content (10%)
- Zeta potential varies slightly*



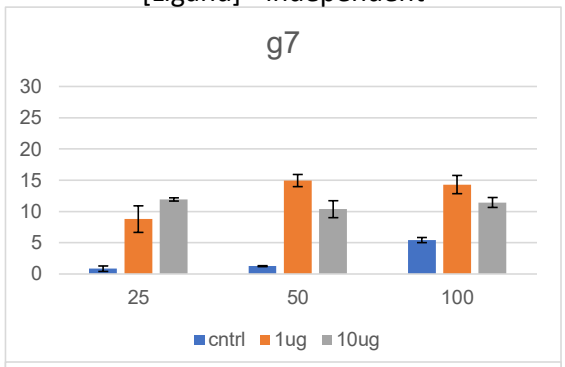
STEM



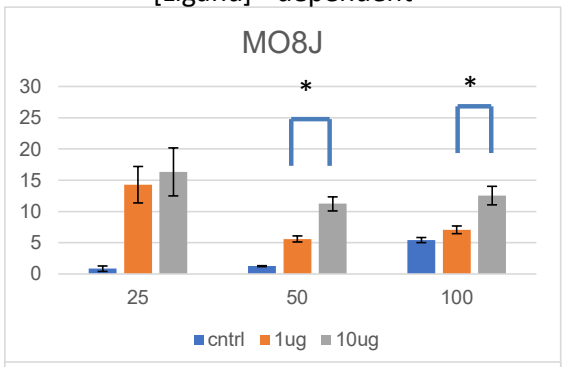
AFM

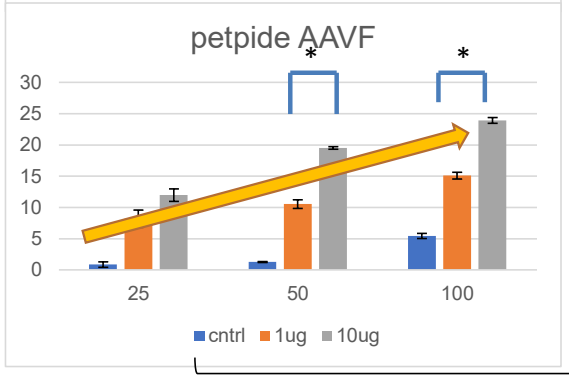
GBM Targeted NMeds: Cell Uptake

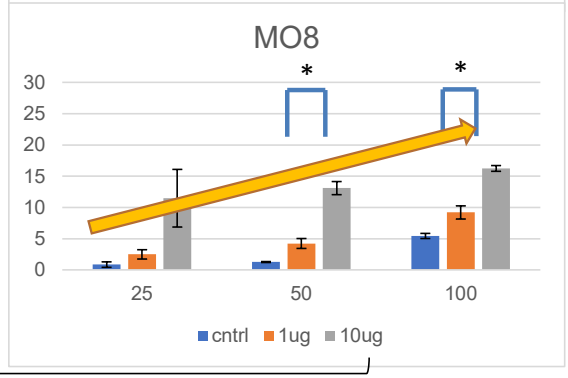
[NP] - independent
[Ligand] - independent



[NP] - independent
[Ligand] - dependent

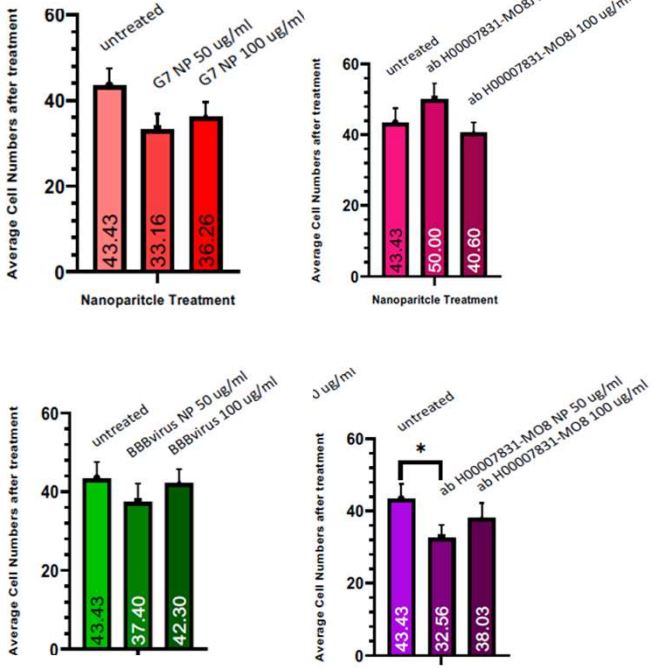




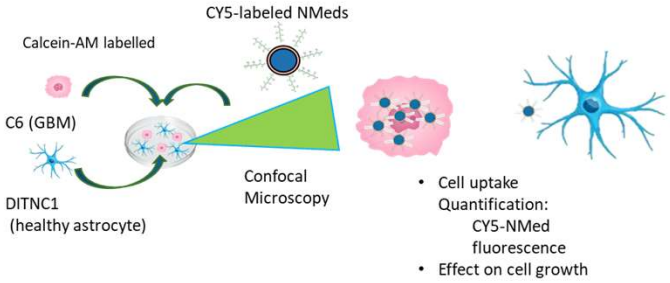


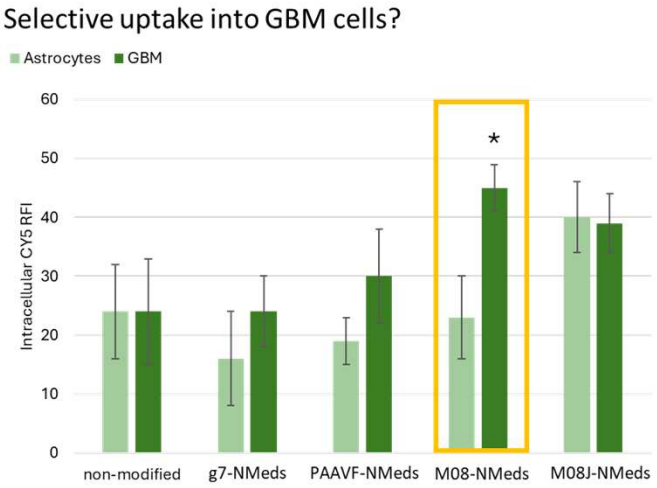
[NP] - dependent
[Ligand] - dependent

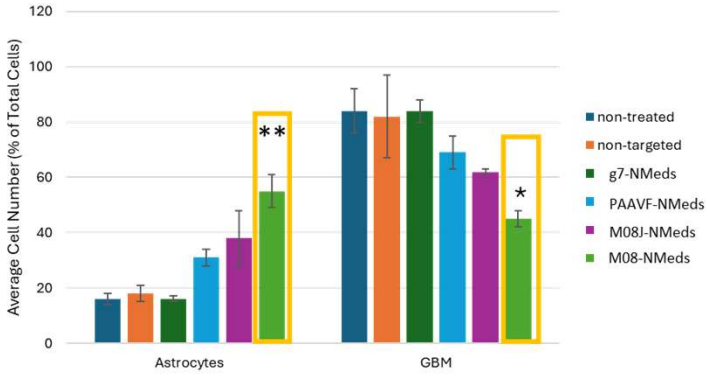
Viability: Cell #



Selectivity: co-culture of C6 (GBM) and DITNC1 (Healthy astrocytes)







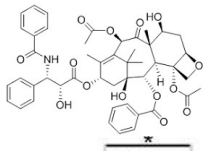
M08 NPs:

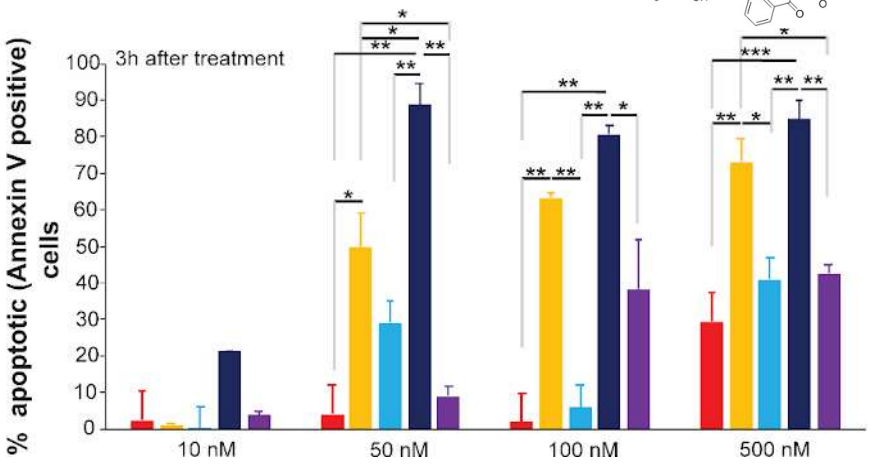
- Selective For C6
- Allows improved Astrocyte #'s
- Potential therapeutic effect?

Paclitaxel-loaded MO8 NMeds

A) Quantification of apoptosis

Annexin V: apoptotic cell marker





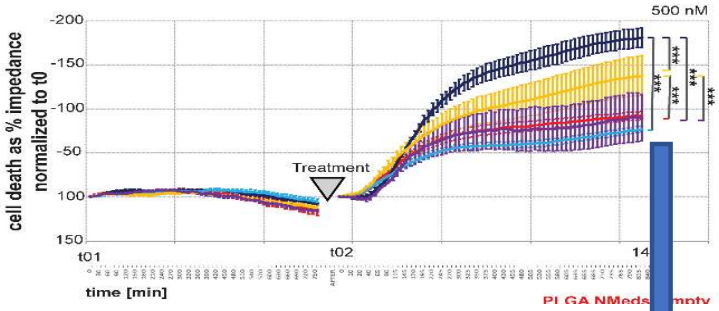
Apoptosis rate compared to Free PTX:

- Increases with concentration
- Non-targeted, Non-loaded NPs show minimal shift towards apoptosis
- Non-loaded MO8-NPs have an effect without PTX
- Non-targeted PXT –NPs increase apoptotic markers

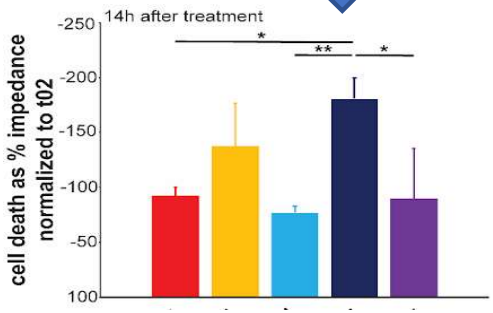
- *MO8-PTX-NPs (50 nM) already appear to saturate already at 50 nM (near 100% of cells show apoptotic markers)

B) Cell Vitality: real time impedence measurements

↓ Impendance = ↑ Cell death



PLGA NMeds_empty
PLGA NMeds_PTX-loaded
PLGA NMeds_empty M08 Ab-targeted
PLGA NMeds_PTX-loaded M08 Ab-targeted
free PTX



PTX-M08 NMeds > Biological effect than all other NMeds

BBB- Targeting

GBM targeting (MO8)

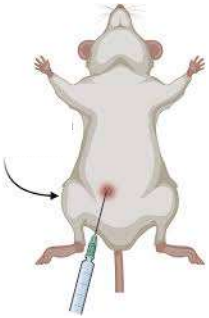
BBB targeting (g7)

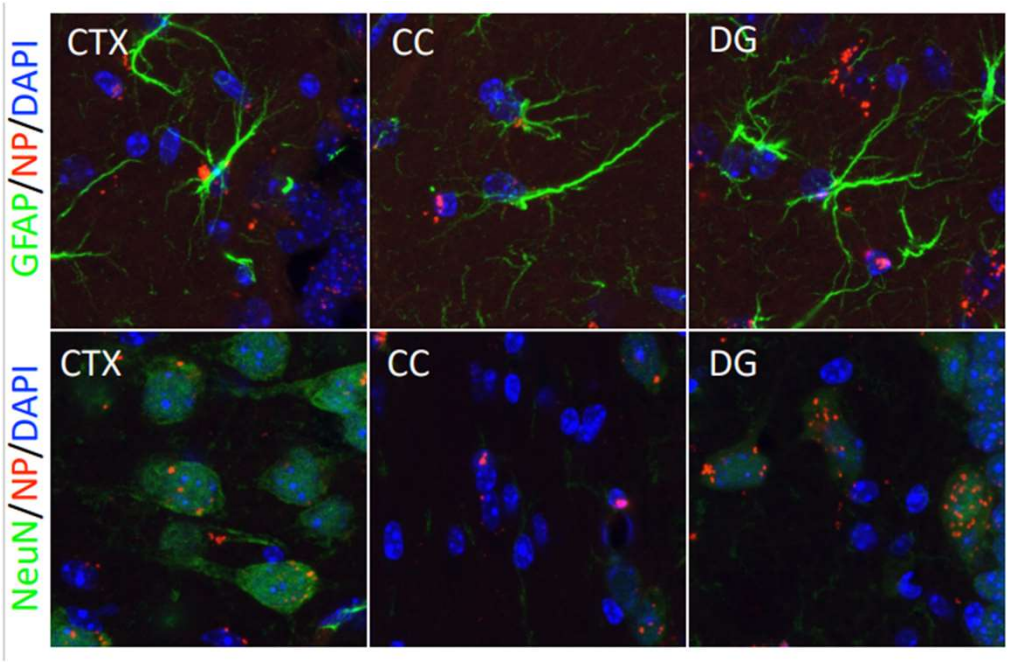
GBM and BBB targeting (g7)

MO8 – PLGA NMeds colocalize with

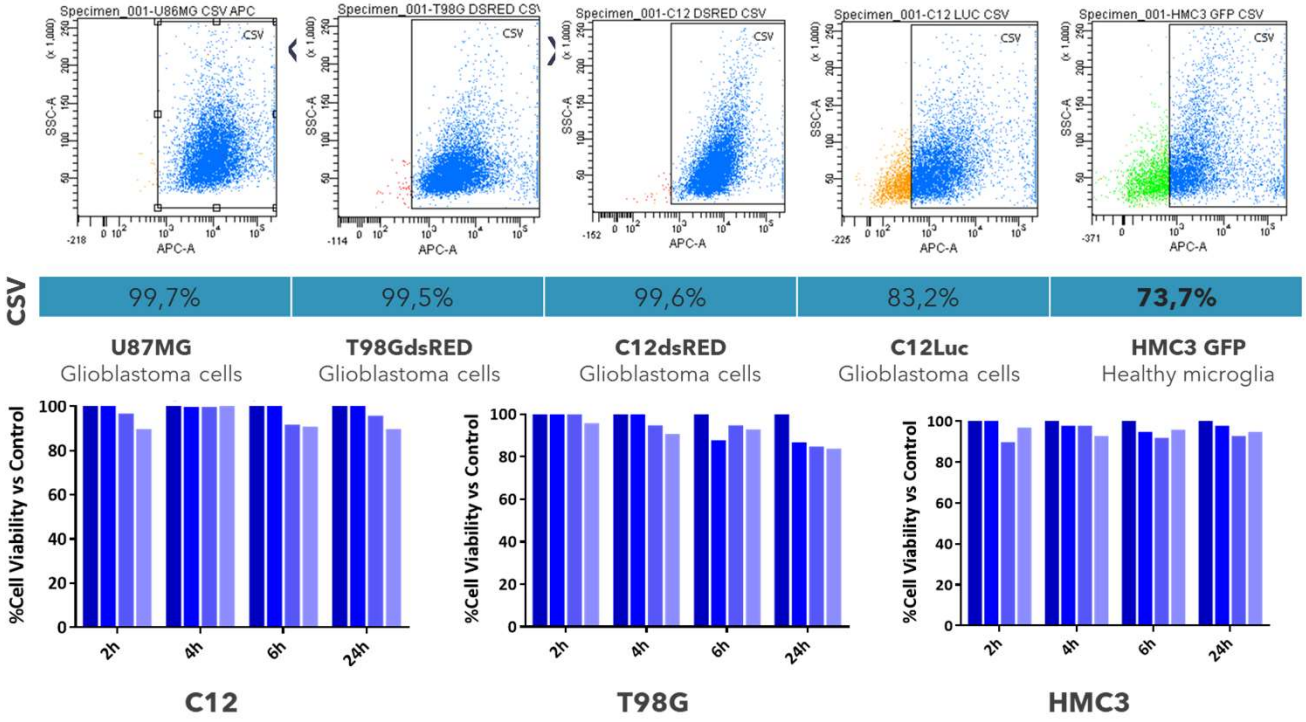
- Neurons (NeuN)
- Microglia (Iba1 not shown)
- NOT with GFAP+ astroglia

C57Bl6J
IP injection 20 mg/Kg
Sacrificed after 6 hours





Advancing Towards The Future: Improved Specificity



MO8 NMeds

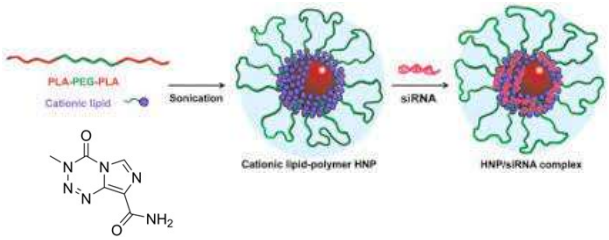
*New Ligand

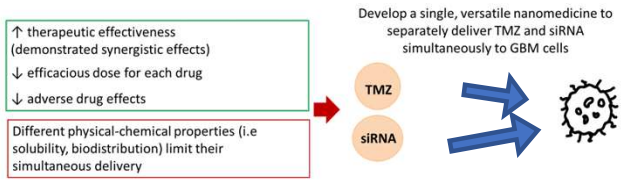
Higher specificity

10% in HMC3 cells

Advancing Towards The Future

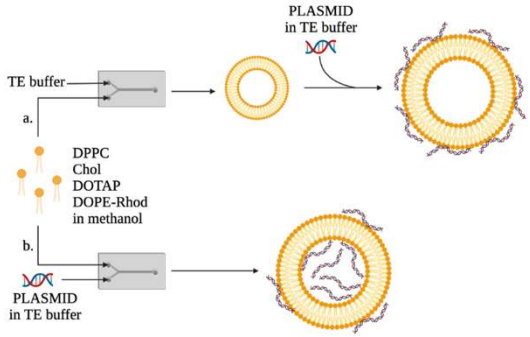
Hybrid particles: co-delivery of siRNA and/or Temozolomide





https://doi.org/10.1016/j.ijpharm.2024.123994

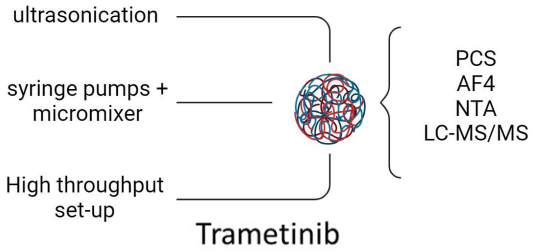
Microfluidic Lipid Particles for: Plasmid DNA – siRNA - mRNA



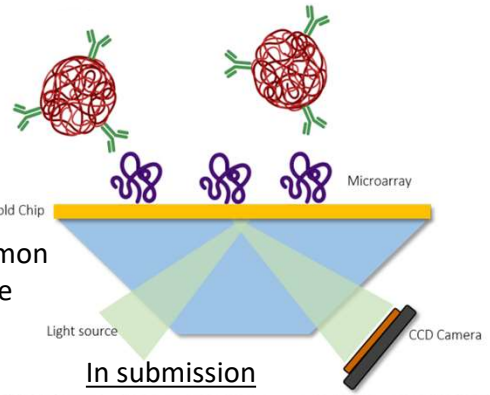
Special Issue in Pharmaceutics

10.2147/IJN.S457302

Advanced characterization and Different Drugs



Trametinib



Surface Plasmon Resonance

In submission

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