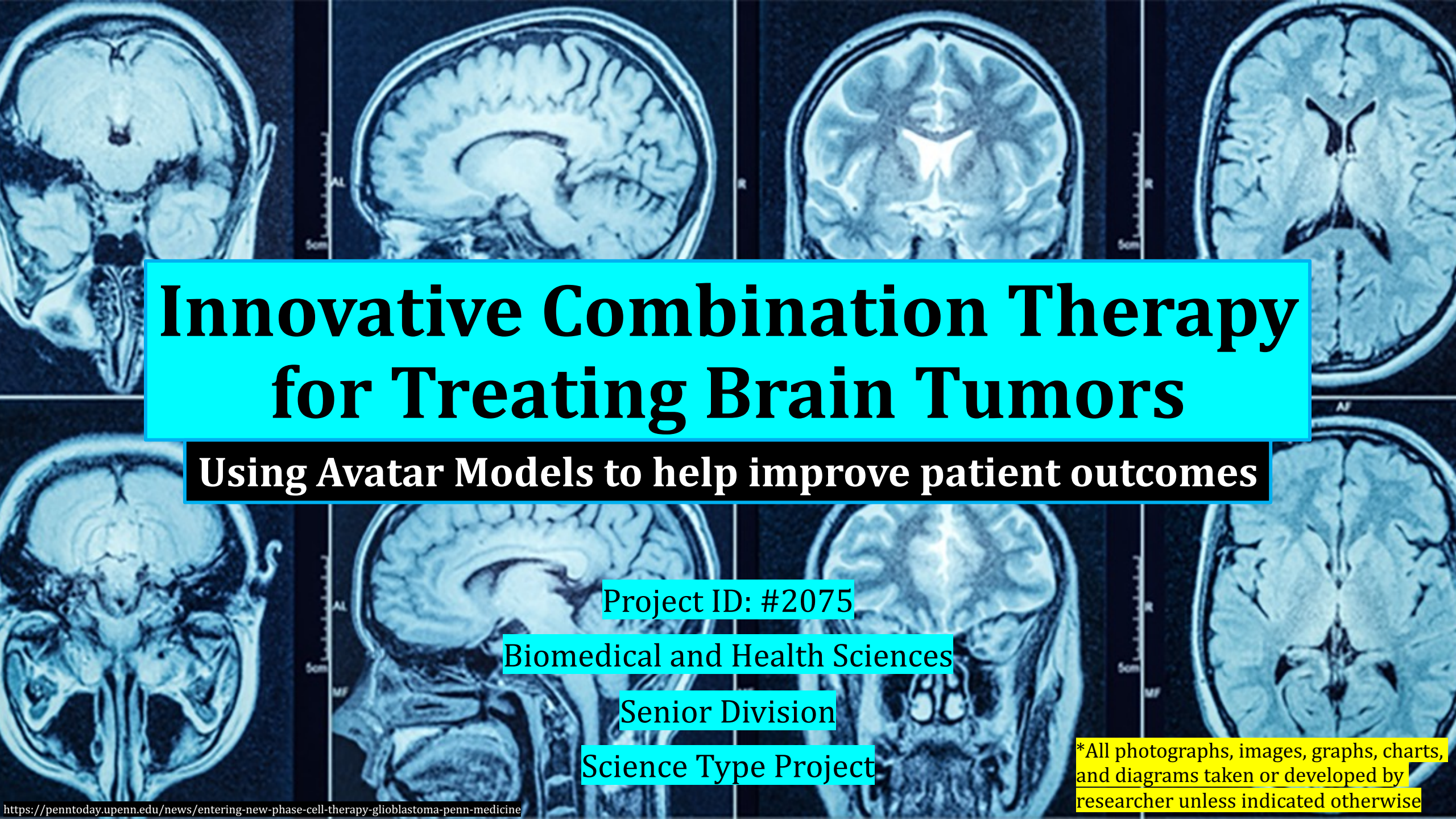


Innovative Combination Therapy for Treating Brain Tumors

Using Avatar Models to help improve patient outcomes

Project ID: #2075

Biomedical and Health Sciences

Senior Division

Science Type Project

*All photographs, images, graphs, charts, and diagrams taken or developed by researcher unless indicated otherwise

https://penntoday.upenn.edu/news/entering-new-phase-cell-therapy-glioblastoma-penn-medicine

Introduction: Glioblastoma Multiforme (GBM)

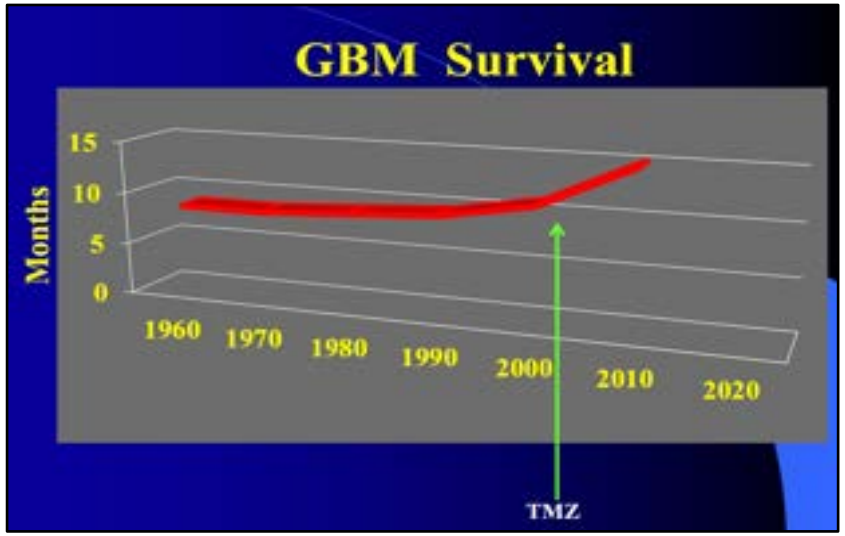
Current Prognosis

I. The most malignant & lethal form of brain cancer

II. Median survival rate is devastating
- With no treatment: <4 months
- With ***optimal*** treatment: <15 months

III. GBM continues to evade advancements in medicine
- Nearly the same dismal prognosis as in 1970 (despite decades of research)

IV. Dire need for superior treatment



Typical Treatment

I. Surgical Resection
- Highly fibrous, interdigitated with normal tissue: impossible to remove 100% of tumor without harming normal brain
- Even after extensive resection, GBM will rapidly grow back

II. Radiation Therapy
- Broad, harmful radiation waves disturb many untargeted areas
- Detrimental effects to healthy tissues

III. Chemotherapy
- Initially somewhat effective, but tumor quickly **acquires chemoresistance,** stops responding to therapy

Proposed Treatment

➢ *Improving* chemo by **inhibiting** acquired chemoresistance

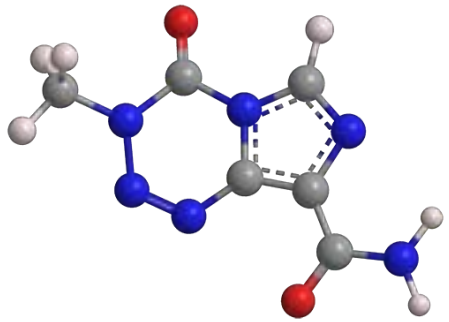
➢ Novel, independently developed **combination therapy** (using Avatar Models)

➢ *Can our proposed combination therapy more effectively treat GBM than the typical monotherapy, temozolomide (TMZ)?*

Proposed 3-Factor Combination Therapy

Temozolomide (TMZ)

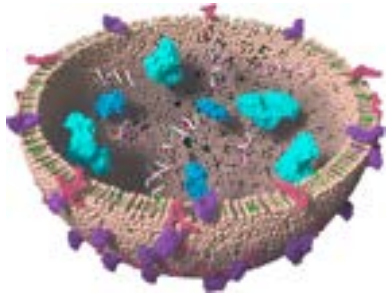
I. Frontline chemo drug to treat GBM
II. Drug resistance
    - Tumor cells will rapidly acquire chemoresistance, making it less effective
III. Administered orally



https://www.acs.org/molecule-of-the-week/archive/t/temozolomide.html

Engineered Exosomes

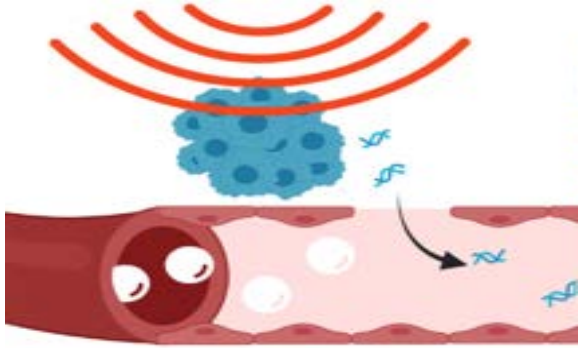
I. CEC-m214-exos, derived from human cerebral endothelial cells, serves as a vehicle to carry micro-RNA to tumor
II. This modified genetic material payload combats tumor's chemoresistance, thereby increasing TMZ efficacy



https://pubs.acs.org/doi/10.1021/acs.chemmater.9b00050

Focused Ultrasound (FUS)

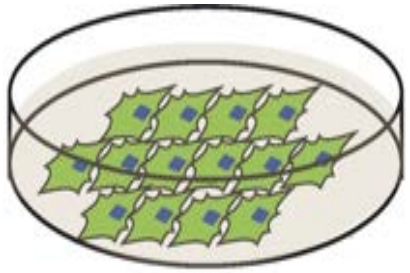
I. High frequency (~20kHz) sonic waves target tissue on blood-brain barrier (BBB) surrounding tumor
II. Entryway opens, exosomes and TMZ enter treatment area in higher volumes
    - Further reduces tumor's chemoresistance



https://localtoday.news/mo/focused-ultrasound-unleashes-brain-biomarkers-137933.html

Proof of Concept -- Methodology, Part I: In Vitro Studies

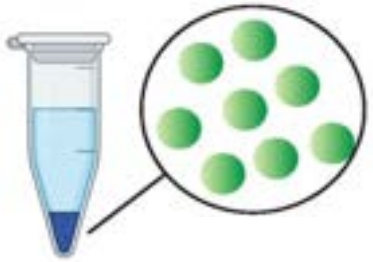
1. Human cerebral endothelial cells transfected with ***miR-214***



Conditioned Medium

2. Differential Ultracentrifuge

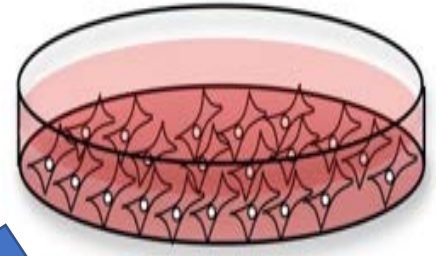
3. CEC-m214-exos



4. Chemotherapy Drug

MicroRNA Delivery

5. GBM Tumor Cells



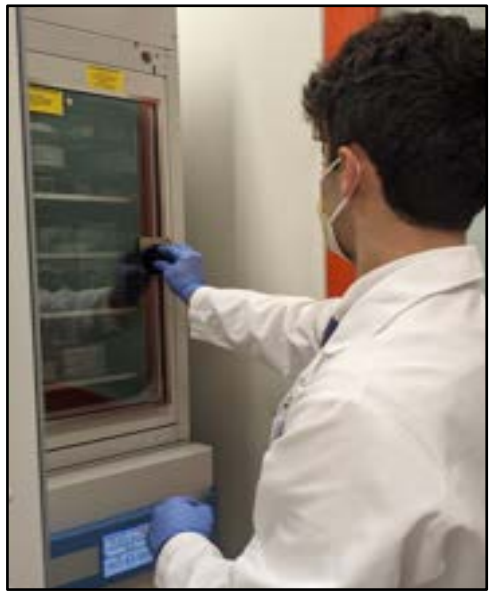


Photo of me utilizing incubator to facilitate tumor cell therapy

Evaluation of tumor cell growth

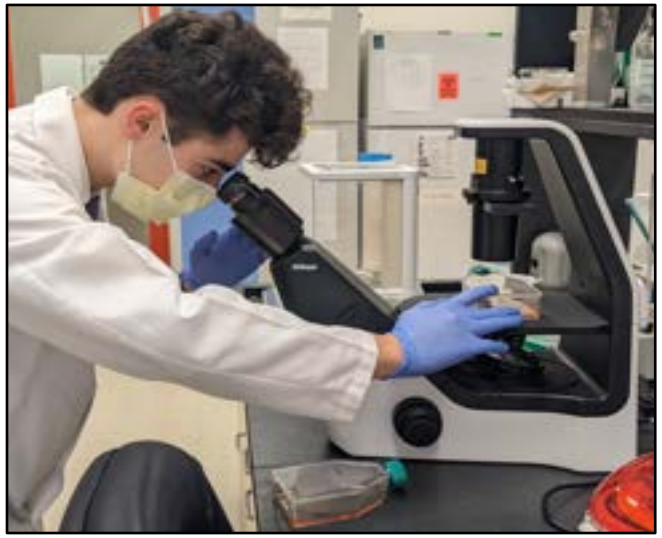
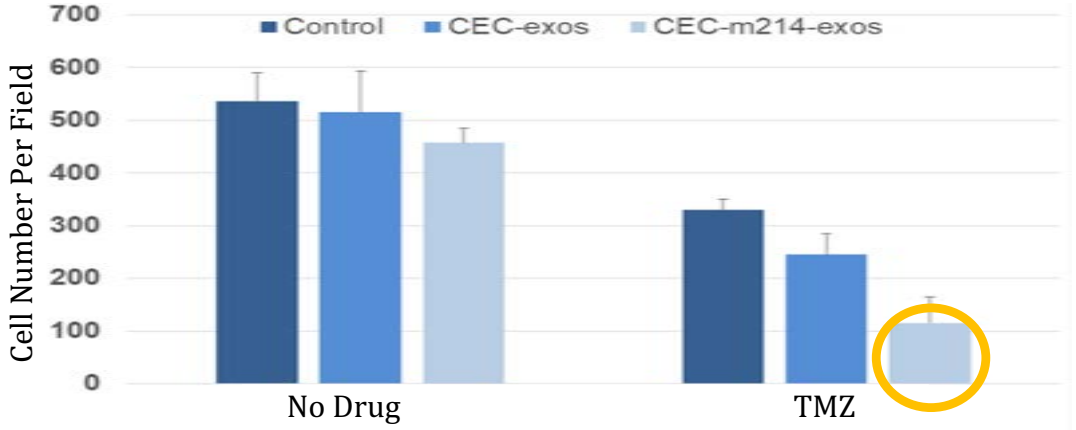
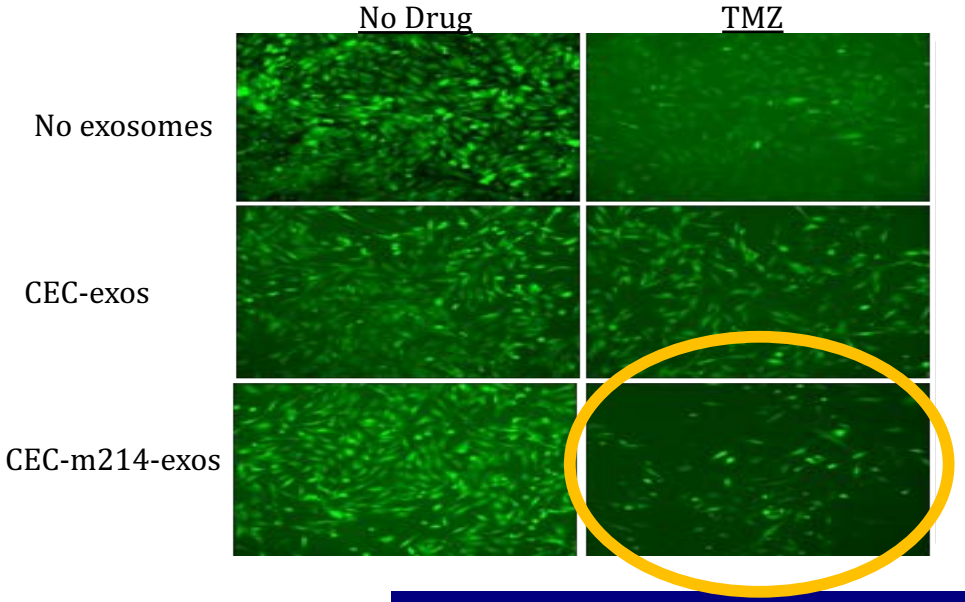


Photo of me analyzing tumor cell growth post-treatment

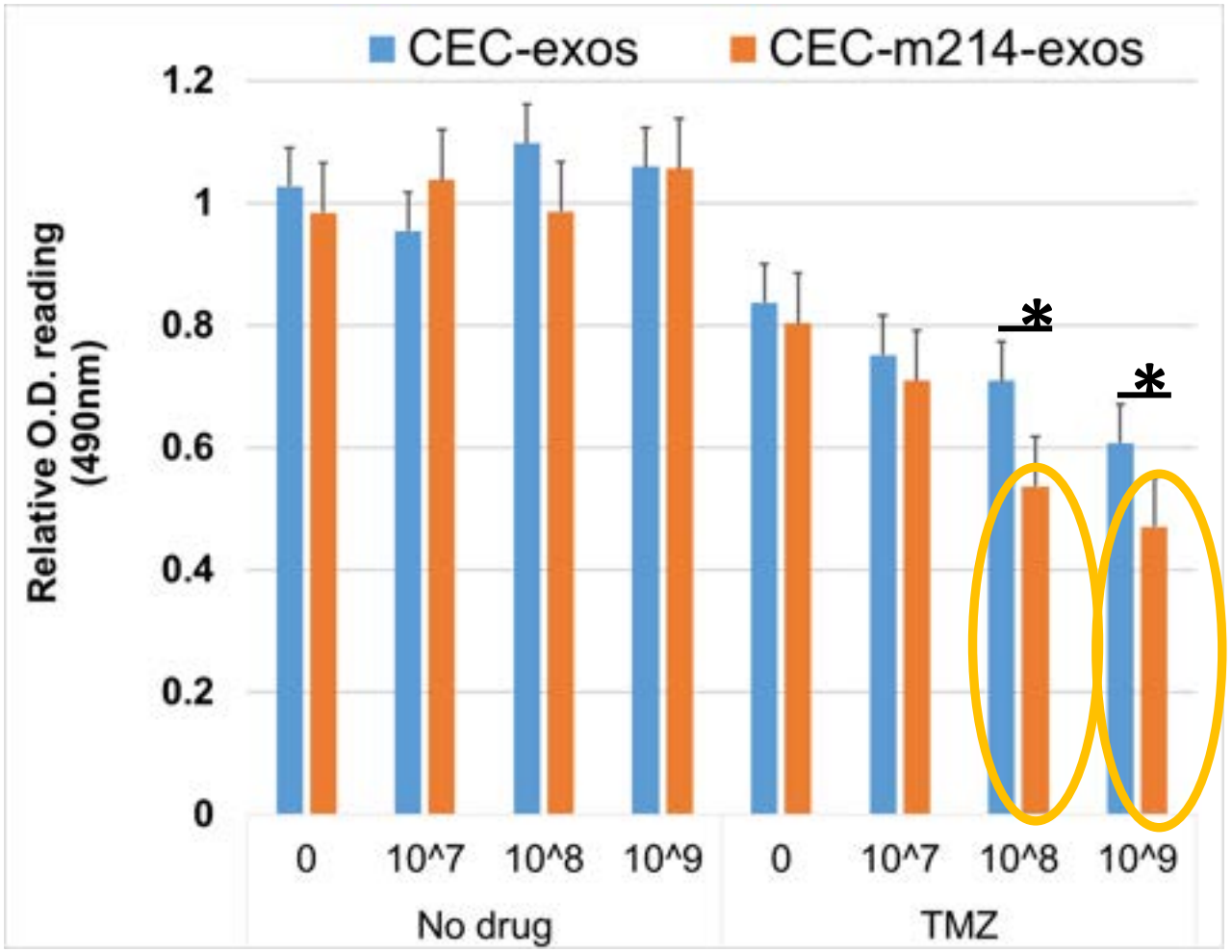
In Vitro Results: Chemoresistance Reduction Analysis

Anti-Invasion Assay – Human GBM Cells





Cell Viability Assay – Human GBM



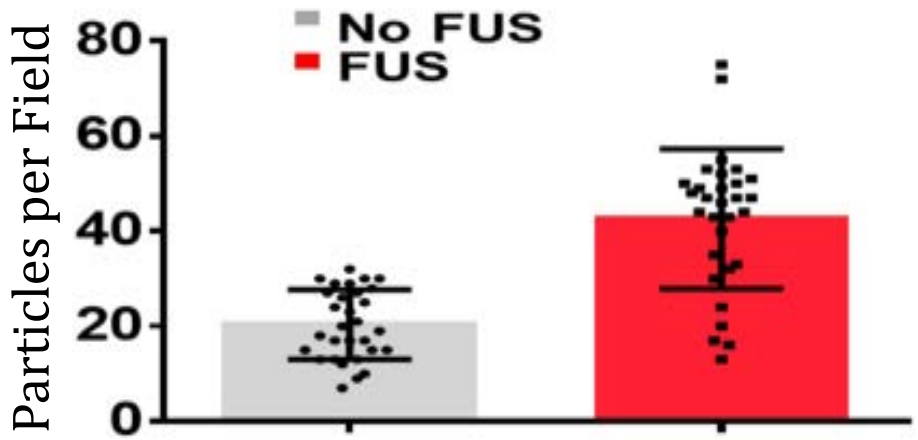
CEC-m214-exos + TMZ increase TMZ efficacy

In Vivo: FUS & Blood-Brain Barrier (BBB), Explained

I. BBB is Central Nervous System (CNS) immunological defense feature
   - Microvascular endothelial cells line capillaries
   - Prevents lipid-soluble substances from passing into CNS tissue

II. Consequently, this prevents efficient drug delivery
   - Inhibits entry of engineered exosomes & TMZ

III. FUS uses high-frequency (~20kHz) sonic waves to clear path through BBB
   - Direct access to tumor for CEC-m214-exos & TMZ



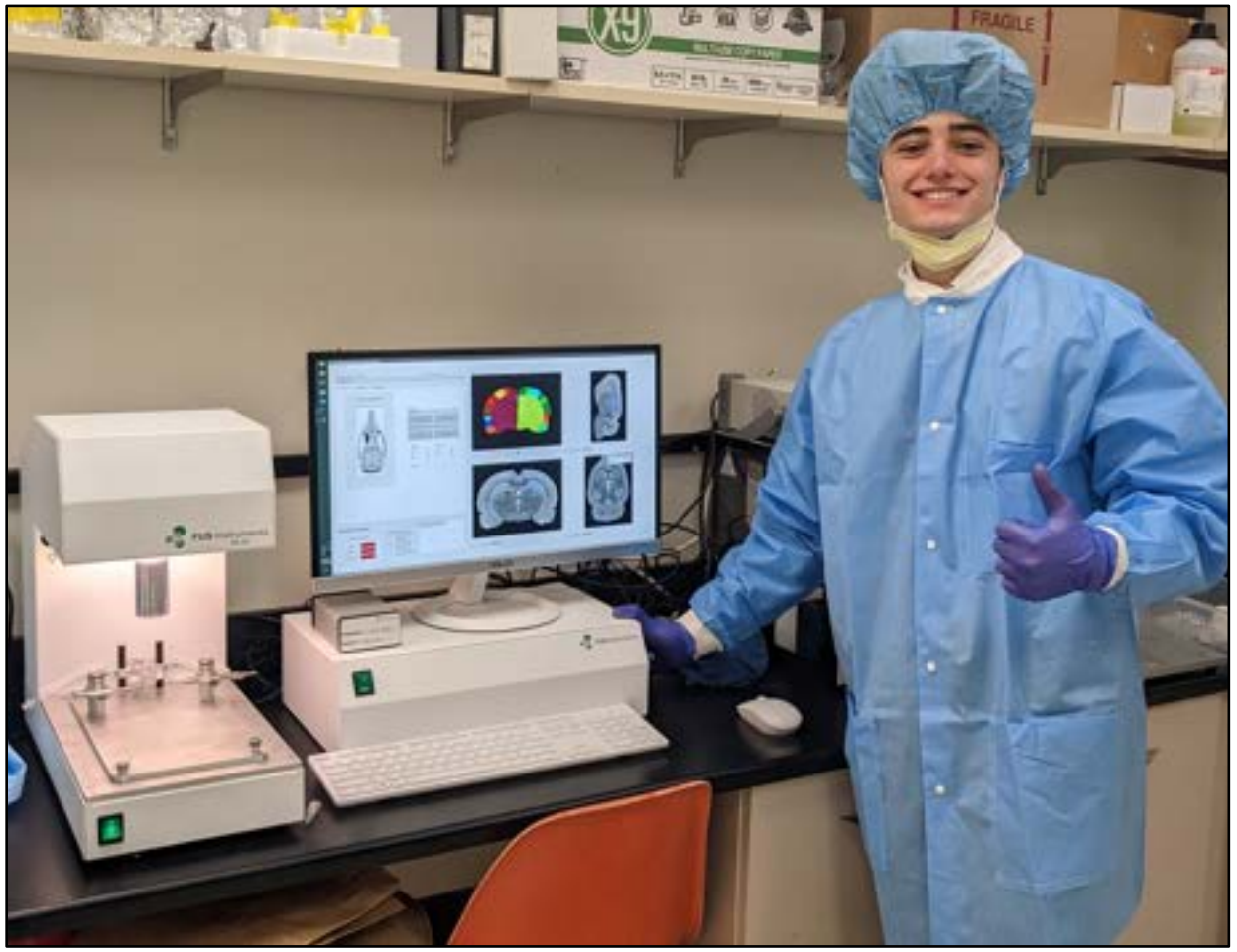


Photo of me analyzing brain scans of FUS and the cross-sections of a GBM-bearing Nude Rat brain

Methodology, Part II: Establishing a Comprehensive Avatar Model

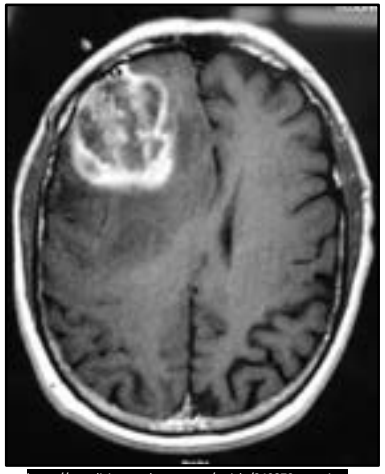
Specific Mammal Model: **Nude Rat** (subspecies of *Rattus norvegicus*)

- Immunodeficient & outbred organisms: ideal for tumor biology, experimental therapy, xenograft research
  - Can manipulate tumor & treatment procedures

Engineering the Avatar: Duplicate manifestation of human patient's GBM, but in another organism

- Grants ability to treat the same GBM (identical biological makeup) in the Nude Rat first
  - Extrapolation of results to human GBM patient

1. Patient with GBM



https://emedicine.medscape.com/article/340870-overview

Resect tissue during surgery

2. Fresh resected tissue
(pathology, consent, clinical data)

https://www.mdpi.com/2409-9279/3/1/11

Enzymatic dissociation

3. Stem cells, progenitor cells, differentiated cells

https://www.123rf.com/clipart-vector/cell_culture_dish.html

Cell culture

4. Neuro-sphere cell selective medium

https://wellcomecollection.org/works/j52swvgv

Intracranial implant

5. Photo of me performing neurosurgery for tumor implantation

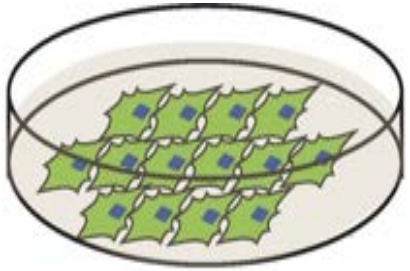
Brief tumor cultivation period

6. GBM-Bearing Nude Rat:
Completed Avatar Model

Methodology, Part III: Administration of Treatment to Avatar Model

- Post-tumor implantation, GBM has taken hold in avatar model
- Administer differing levels of combination therapy, observe and measure
- Utilized measurement methods:
  - MRI brain scans – *dynamically* measure tumor & lesion
  - Resected tumor slices – tumor volume
  - Longer-term survival rate study

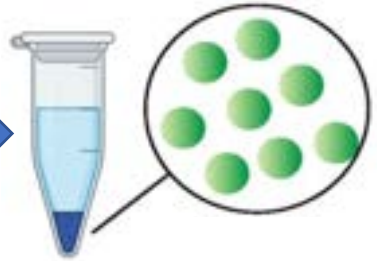
1. Human cerebral endothelial cells transfected with ***miR-214***



Conditioned Medium

2. Differential Ultracentrifuge

3. CEC-m214-exos



4. FUS

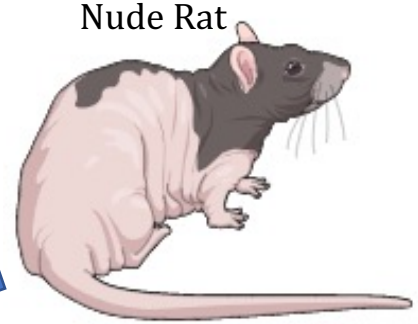
Open BBB for exosome entry to tumor

5. TMZ

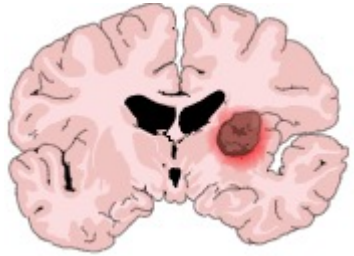
Oral administration

Intranasal administration

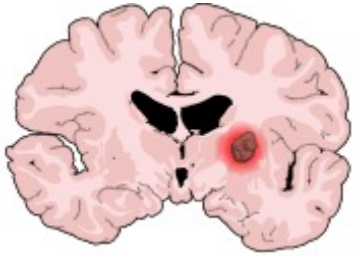
6. GBM-Bearing Nude Rat



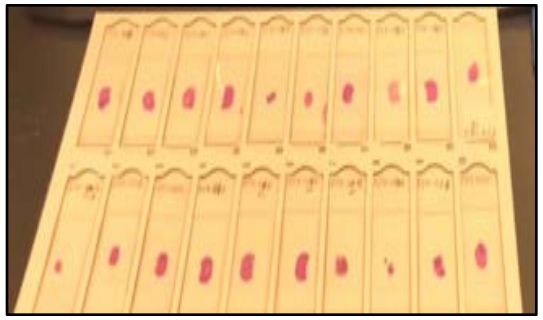
Brain scan imaging



Further imaging shows tumor volume reduction

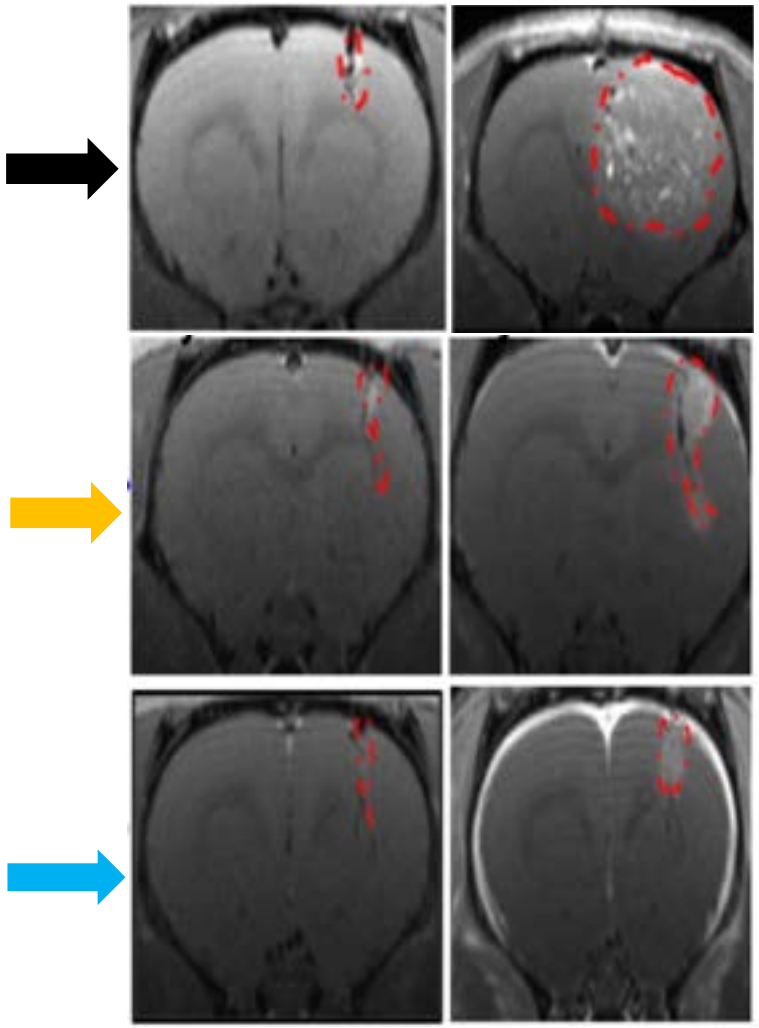


Tumor slice segmentation for volume analysis



In Vivo Results: Brain MRIs & Tumor Volume

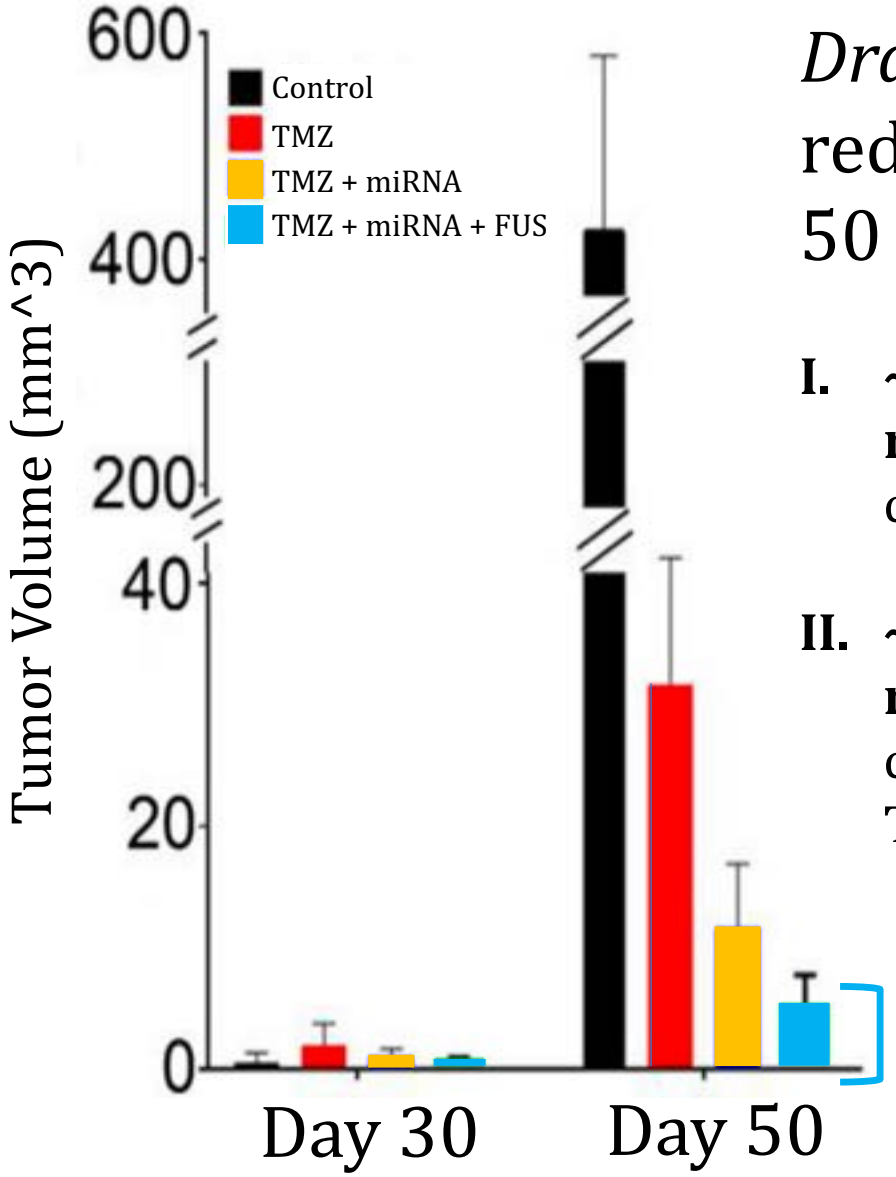
Day 30	Day 50



Control: no treatment

TMZ + miRNA

TMZ + miRNA + FUS



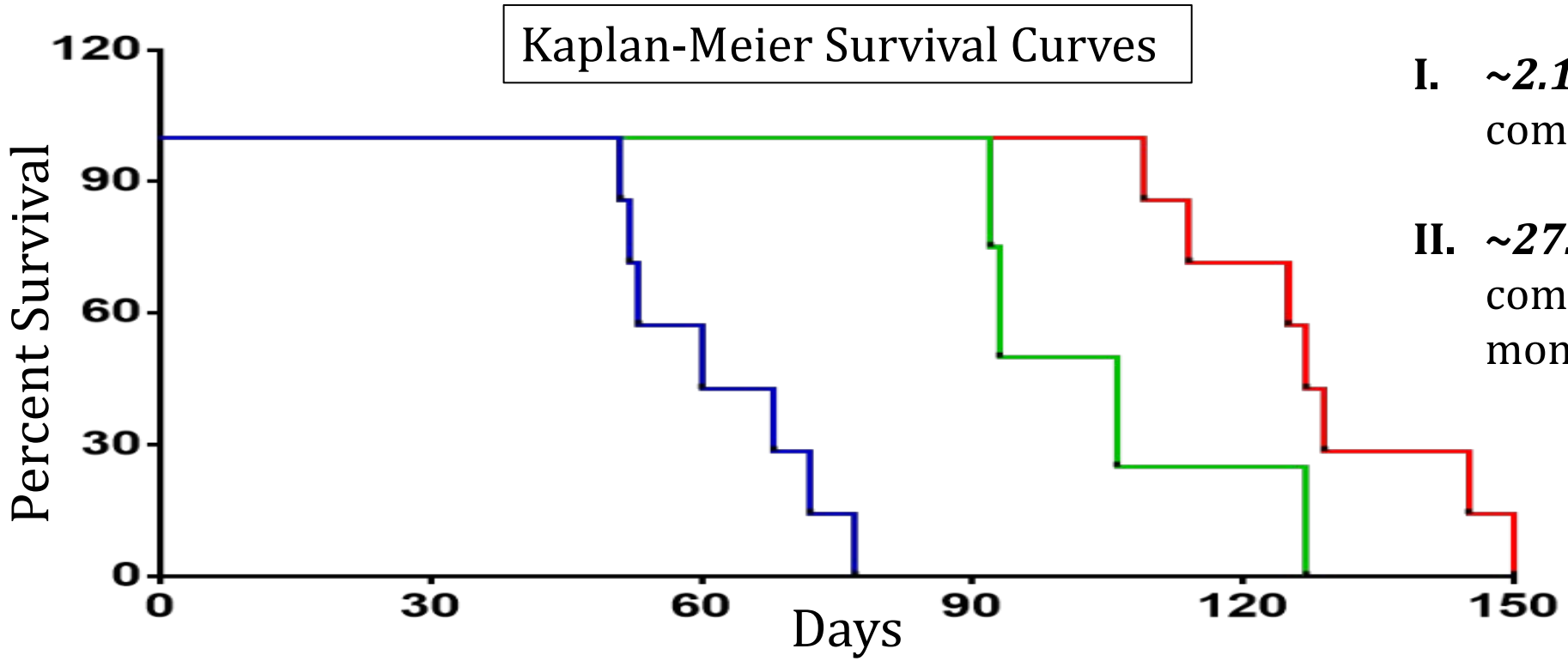
Drastic tumor reduction after 50 days:

I. **~*77x* volume reduction** compared to control

II. **~*6x* volume reduction** compared to typical TMZ monotherapy

In Vivo Results: Survival Rate Analysis

- Repeated procedure across data set of 8 rats, measured survival times
- Control = no treatment
- Green = TMZ only (typical treatment)
- Red = TMZ + CEC-m214-exos



	Control	TMZ	TMZ+214
Median Survival Time(Day)	60	99.5	127

Dramatic increase in survival time:

I. ***~2.12x* as long lifespan** compared to control

II. ***~27.6%* longer lifespan** compared to typical TMZ monotherapy

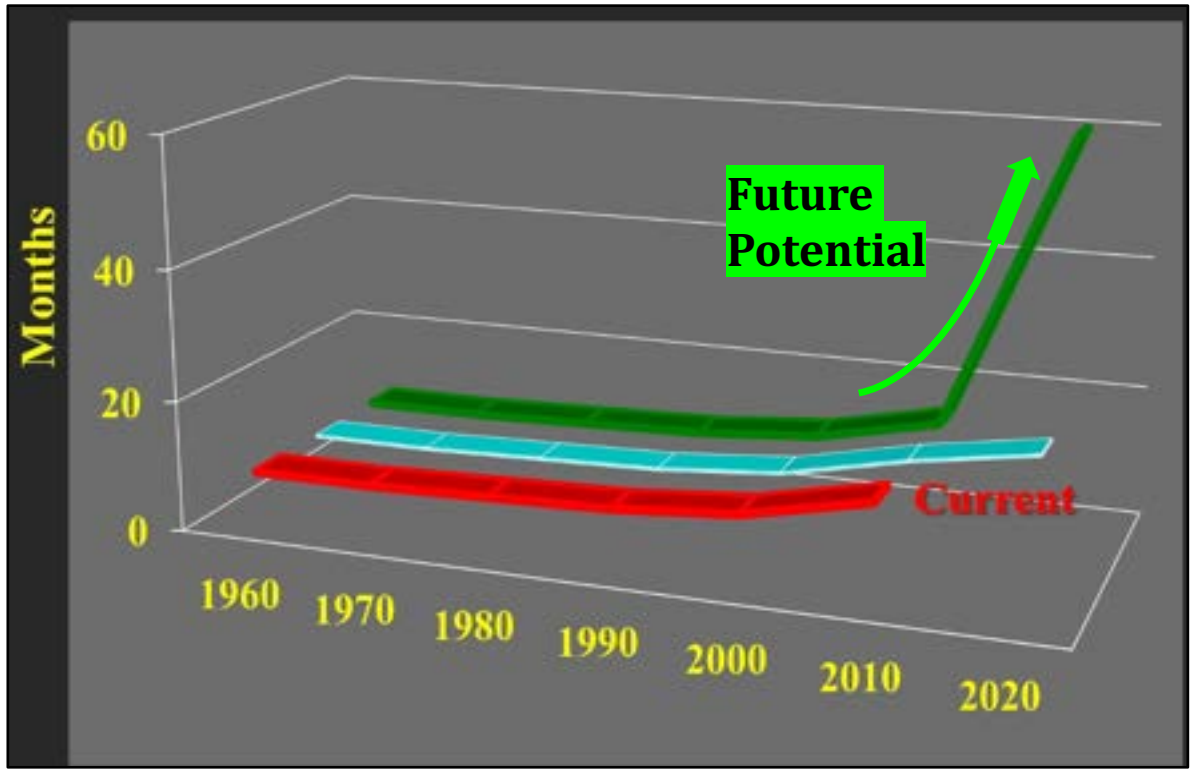
Conclusions & Translating Results

I. Improvements by tumor volume
- TMZ + miRNA + FUS
  - ***77x*** volume reduction compared to control
  - ***6x*** volume reduction compared to current standard (TMZ monotherapy)

II. Improvements by survival rate
- TMZ + miRNA
  - Lifetime ***doubled*** compared to control
  - ***~28%*** longer lifetime compared to current standard (TMZ monotherapy)

III. Avatar Model establishes credible scientific underpinning for translational oncology

GBM treatment: *What Will the Future Hold?*
- This therapy can progress to clinical trials, offer more hope to patients
- Avatar Modeling offers patients individualized approach to GBM treatment



References

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