The Impact of Your Philanthropy

SebastianStong Foundation
July 2023

Fueling Hope for DMG Patients through a Trailblazing Partnership

Kids with deadly diffuse midline gliomas (DMG) have no time to lose. They need cures today. Children's National Hospital is proud to partner with the SebastianStrong Foundation to discover new therapies and save lives. Your community's support is fueling drug discovery efforts that promise to maximize the revolutionary potential of ultrasound technology.

A $672,000 gift in 2022 launched a research collaboration between Children's National, Columbia University and Virginia Tech. We present this six-month progress update with deep appreciation.

Members of the research team investigating potential cures for DMG met at Virginia Tech this spring (L to R): Ayda Woldegerima, Dr. Jennifer Munson, Dr. Javad Nazarian and Sridevi Yadavilli.

Dr. Nazarian (left) and Dr. Eli Vlaisavljevich (right) of Virginia Tech thank the SebastianStrong Foundation for investing in pioneering DMG research.

Studies Launched and On Track

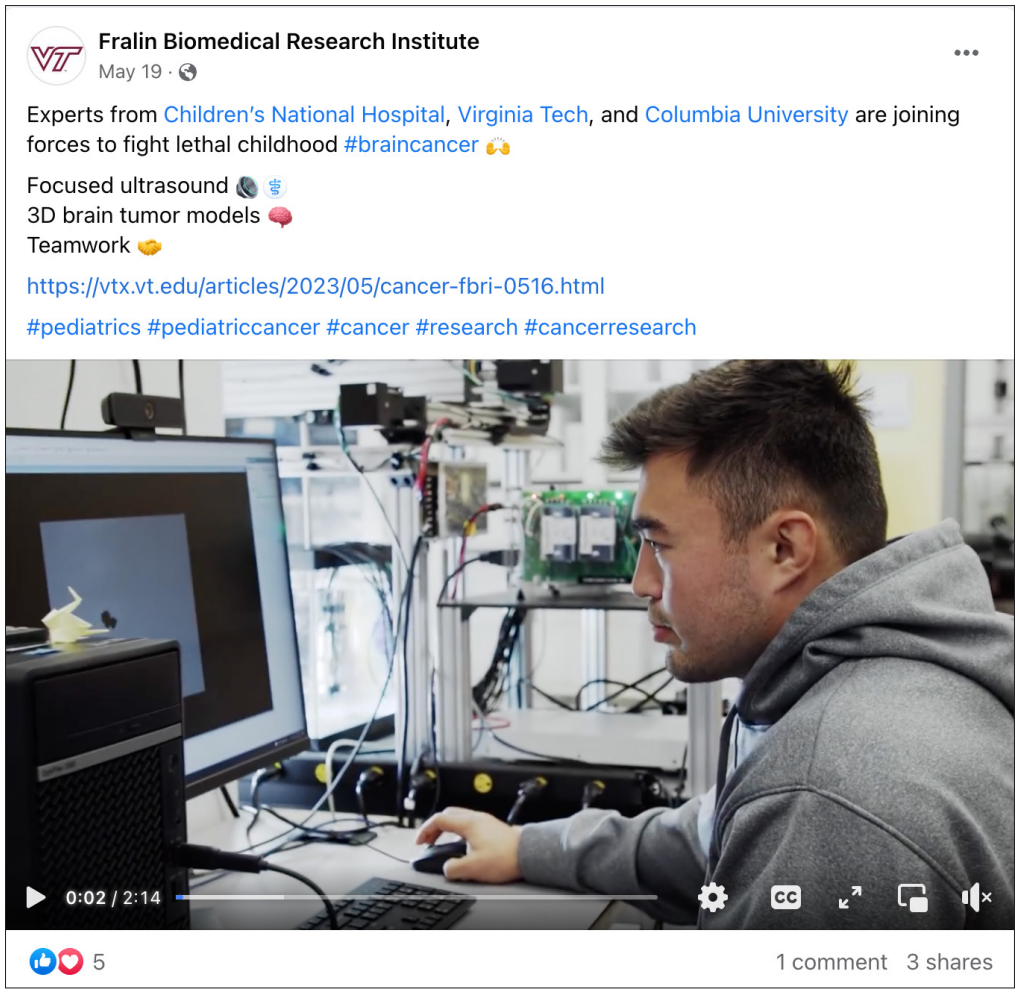
In 2022, Children's National performed the world's first pediatric surgery for a diffuse intrinsic pontine glioma using low intensity focused ultrasound (LIFU) in combination with 5-aminolevulinic acid (5-ALA) medication. The procedure uses LIFU to open the blood-brain barrier non-invasively.

Your gift fast-tracked the testing of additional drugs to use in combination with LIFU. The research team presented its progress in May 2023 at Virginia Tech. Please refer to the attached presentation for technical details and illustrations.

The team's progress includes the following milestones:

- Identifying biomarkers to study the effectiveness of ONC201, a therapy that may hold promise for DMG patients, in combination with LIFU
- Collaborating with Virginia Tech to develop and study novel 3-D DMG in vitro models
- Integrating DMG pre-clinical knowledge with engineering expertise of Virginia Tech
- Optimizing the platform for biomarker study

"We are optimizing biomarker testing in mouse models," says Javad Nazarian, Ph.D., the study's principal investigator. "This will help determine whether LIFU helps enhance the drug's penetration and efficacy. We hope to see breakthrough results."



The Power of Collaboration

The partnership between Children's National, Virginia Tech and Columbia University is laying the groundwork for discovery. Eli Vlaisavljevich, Ph.D., of Virginia Tech, is developing focused ultrasound devices to conduct forthcoming experiments. Jennifer Munson, Ph.D., of Virginia Tech, is creating 3D models. These will give our team a clearer window into how the treatments perform. Cheng-Chia Wu, M.D., Ph.D., of Columbia University, will leverage this research to inform new clinical trials. Dr. Wu previously developed the world's first clinical trial using focused ultrasound in children with relapsed DMG.

Our Gratitude

We remain grateful for everything you do in Sebastian's memory and for every patient with DMG and other deadly childhood tumors. Thank you for racing to cure childhood cancer.

Children's National

Focused Ultrasound Consortium for Treatment of Children Diagnosed with Diffuse Midline Gliomas

Children's National Hospital Center for Genetic Medicine Research | J Nazarian, C. Wu, J. Munson, E. Vlaisavljevich

What's the Plan and Where We Are So Far…

SStrong Focused Ultrasound Project

	Timeline/Milestone Overview	Year.2023				Year. 2024			
		Start Date							End Date
	Aim, Tasks, Subtask, and Milestone Description	Jan - Mar	Apr - Jun	Jul - Sep	Oct - Dec	Jan - Mar	Apr - Jun	Jul - Sep	Oct - Dec
	Aim 1: Establish FUS Sonodynamic benefits for treatment of patients diagnosed with DMG								
1	***Task 1: Assess Biomarkers of response upon FUS + ONC201 treatment***		■	■	■	■	■	■	■
1a	Subtask 1a: Establish Experimental Scheme for biomarker validation post FUS + ONC201		■						
1b	Subtask 1b: Establish Experimental groups/timepoints for murine models of DMG for biomarker validation post FUS+ONC201		■						
1c	Subtask 1c: Optimize platforms for biomarker validation		■						
1d	subtask 1d: Validate Biomarkers of response in murine models of DMG post FUS + ONC201								
	Aim 2: Define clinical FUS to induce immune system engagement in cold tumors including DMGs								
2	***Task 2: Characterize the immunogenic effects of FUS with ICI for DMG***			■	■	■	■	■	■
2a	Subtask 2a: Establish Experimental Scheme for validation of anti-PD1 delivery post FUS								
2b	Subtask 2b: Optimize platforms for validation of anti-PD1 delivery								
2c	Subtask 2c: Analyze Serum and brain tissue with ELISA/Western for anti-PD1 in murine models of DMG treated with FUS+ICI								
3	***Task 3: Assess local changes in transcriptome post FUS treatment***				■	■	■	■	■
3a	Subtask 3a: Establish Experimental Scheme for assessing changes in transcriptome post FUS								
3b	Subtask 3b: Perform RNAseq on murin models of DMG treated with or without FUS to assess sonodynamic effects								
3c	Subtask 3c: Identify local changes in transcriptome post FUS treatment								
4	***Task 4: Characterize the immunogenic effects of FUS + RT with ICI for DMG***					■	■	■	■
4a	Subtask 4a: Establish Experimental Scheme for biomarkers validation post FUS+RT+anti-PD1								
4b	Subtask 4b: Optimize platforms for validation of biomarkers post FUS+RT+anti-PD1								
4c	Subtask 4c: Assess serum and CSF for Methylation Status test for potential biomarkers of response								
4d	Subtask 4d: Perform histological and molecular assays to probe for predictive biomarkers of response post FUS+RT+anti-PD1								

■ Proposed timeline
■ Completed
■ in Progress

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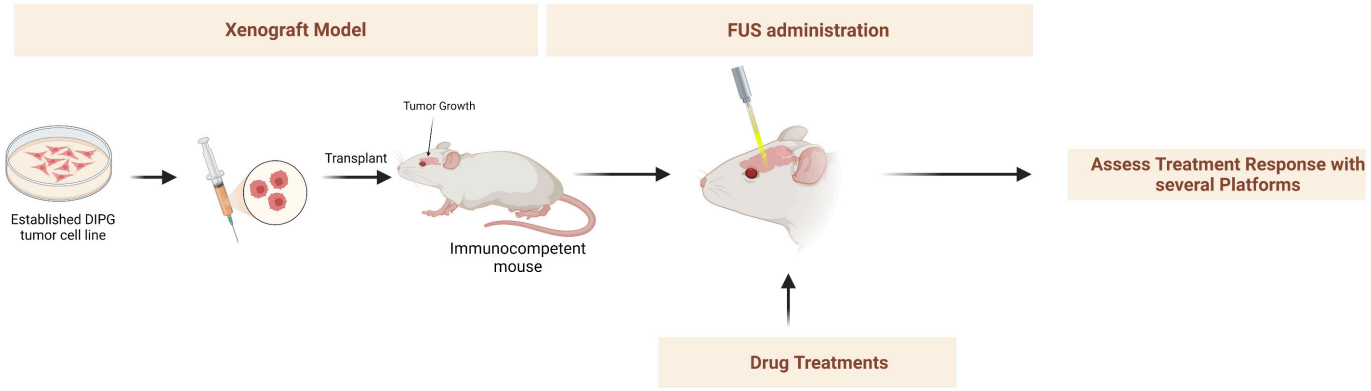
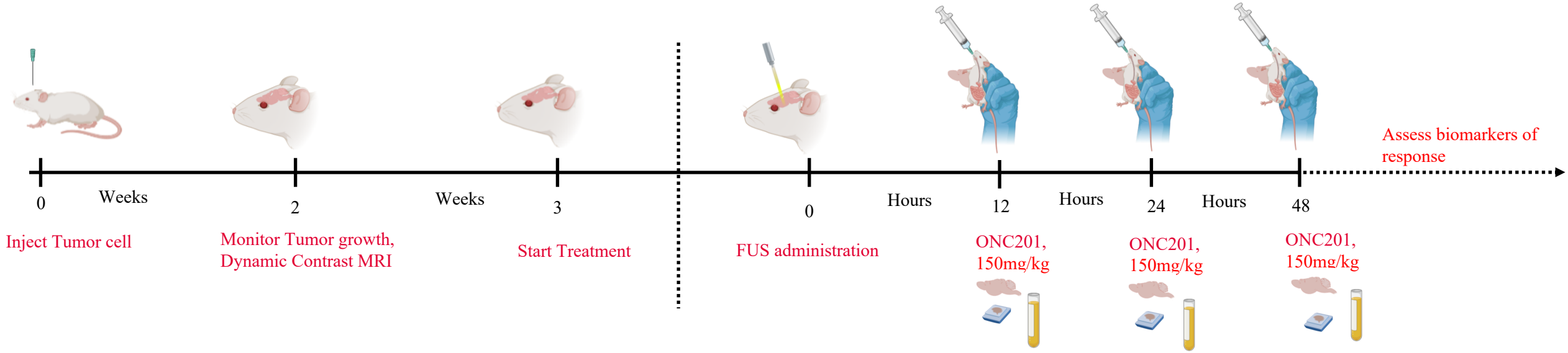
https://cnmc.sharepoint.com/:x:/s/GRP_FUSConsortium_ResearchCtrforGeneticMed/EXga2BpF_exMhTlOs-LLRv8BAteNG0lHbAg8is2Kv2ajHw?e=akuT5n

Biomarker Analysis Post FUS + ONC201 Treatment in Murine Models of DMG



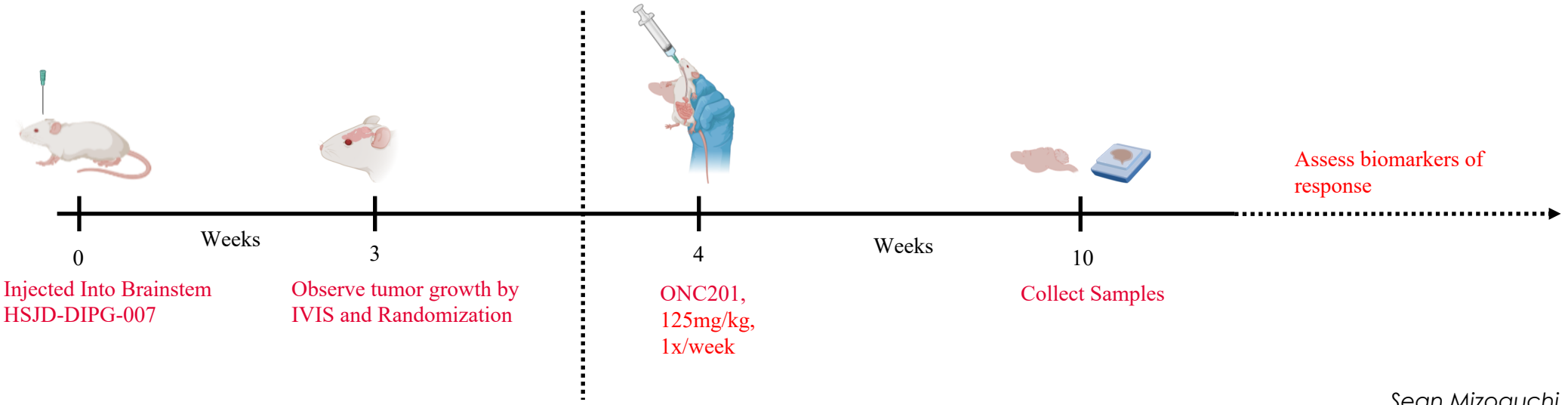
Timeline



Biomarker Analysis

- 4 Experimental arms
  - Control, ONC201, FUS and ONC201+FUS
- Total Mice = 16
  - 4 mice per arm – 1 per timepoint
- Samples collected will be Plasma, frozen Brain and FFPE
- Samples will be sent to Children's National Research Institute early June

Optimization of Biomarker Analysis Post ONC201 Treatment



Sean Mizoguchi

HSJD-DIPG-007

- Patient derived Diffuse intrinsic pontine glioma cell line
- H3.3K27M Mutation
- ACVR1 Mutation

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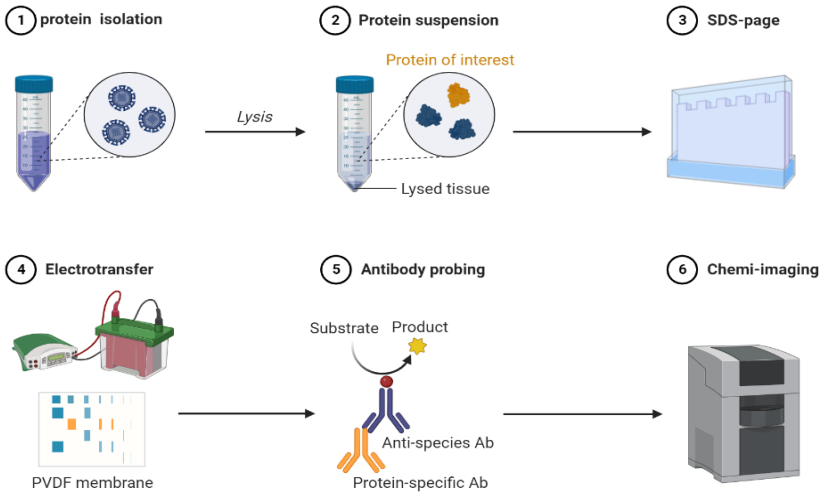
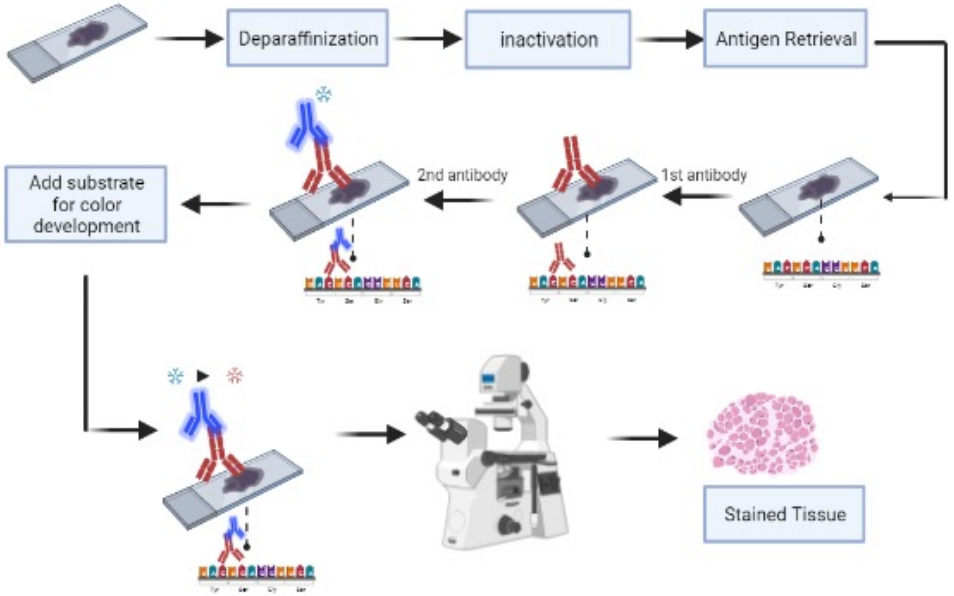
Biomarker Analysis

Western Blot



IHC



Biomarkers to assess:

- ClpP
- ClpX
- NUDAF12
- ATF4
- CHOP
- BCL2
- Cleaved Caspace-3

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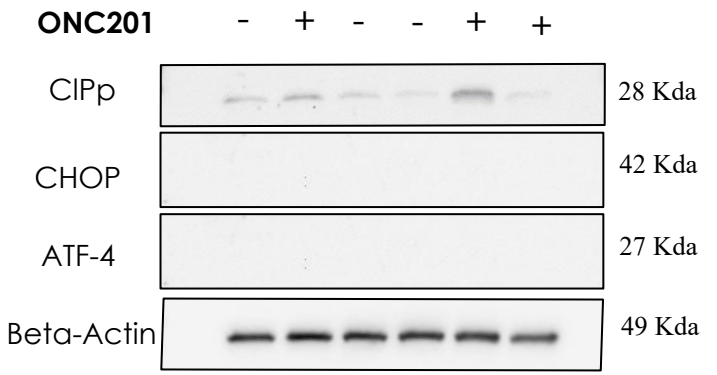
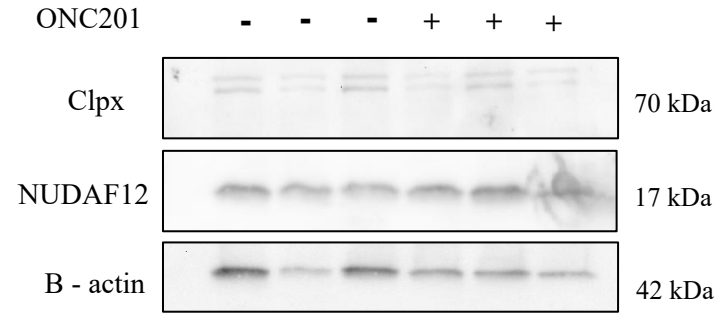
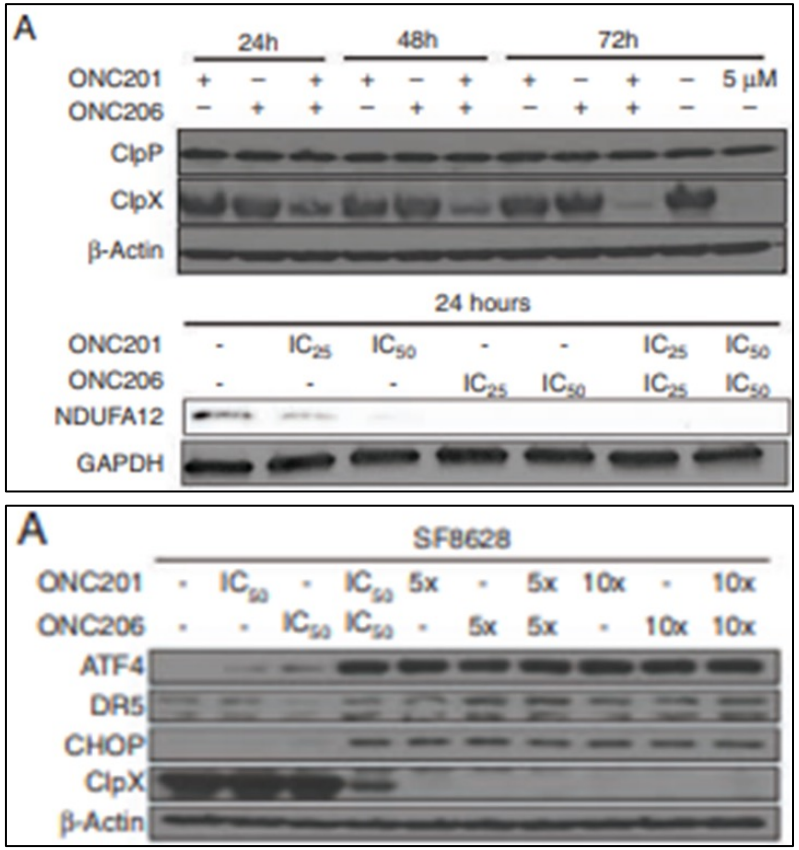
Biomarker Analysis Post ONC201 Treatment in Murine Models of DMG







Przystal et al, Neuro-Oncology 2022

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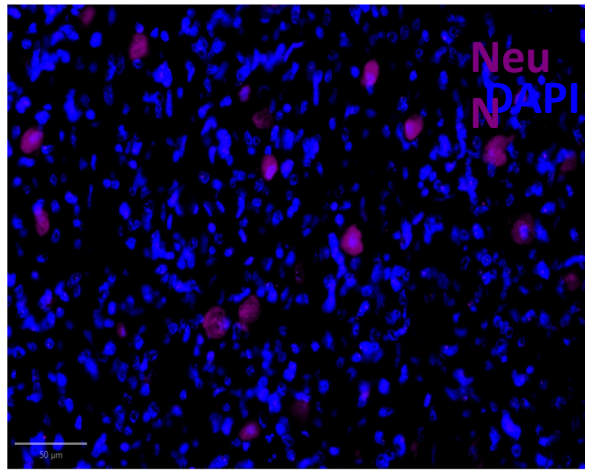
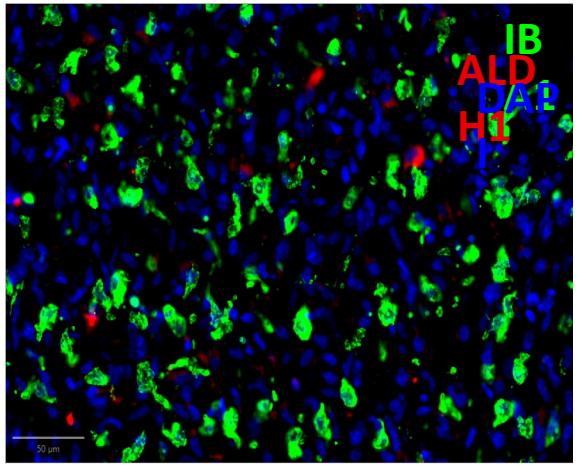
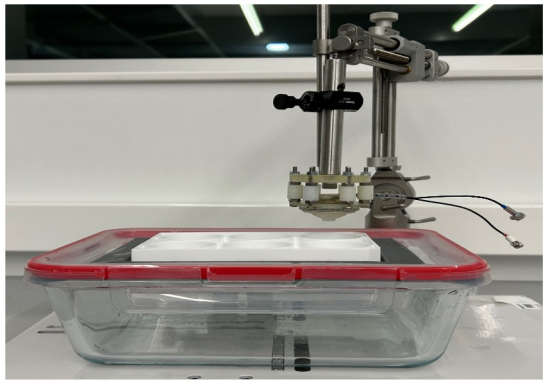
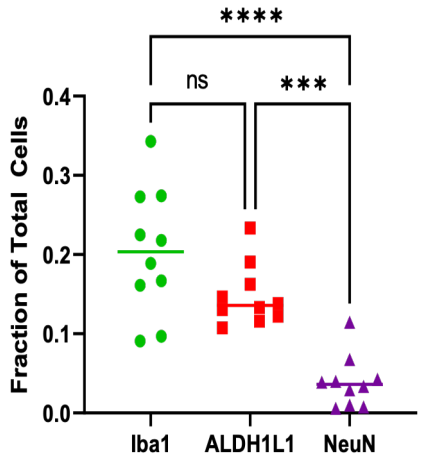
Development of an In vitro Model of DMG



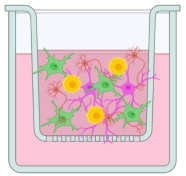


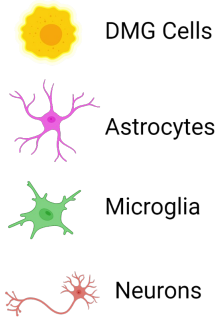
In vitro FUS system

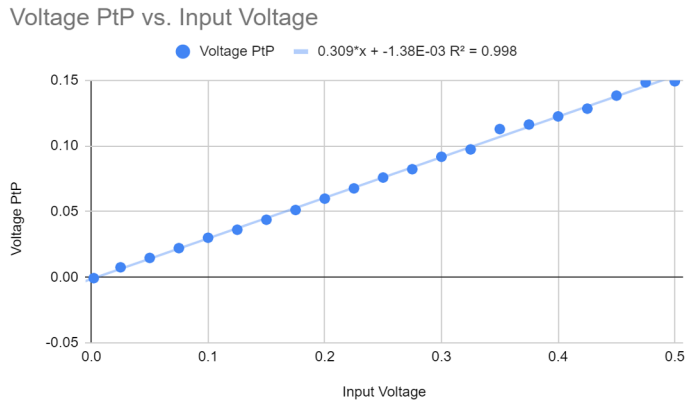




Quantification of cell types in patient samples of diffuse midline glioma.







Next Steps

- Optimization of IHC with murine models of DMG post ONC201 treatment

- Validation of Biomarkers in Patient Tissue post ONC201 treatment

- Validation of Biomarkers in murine models of DMG Post FUS+ONC201

Project 2: Define Clinical FUS to Induce Immune System Engagement in Cold Tumors Including DMGs

Aim 2: Characterize the immunogenic effects of FUS and RT with ICI for DMG

- Testing feasibility of enhanced anti-PD1 delivery with FUS

Experimental Design

Anti-PD1 alone Anti-PD1 + FUS

Specimen	Assay	Read-out
Tissue(50mg)	Western Blot	Anti-PD1
Non-invasive imaging	Optical label anti-PD1	Imaging finding

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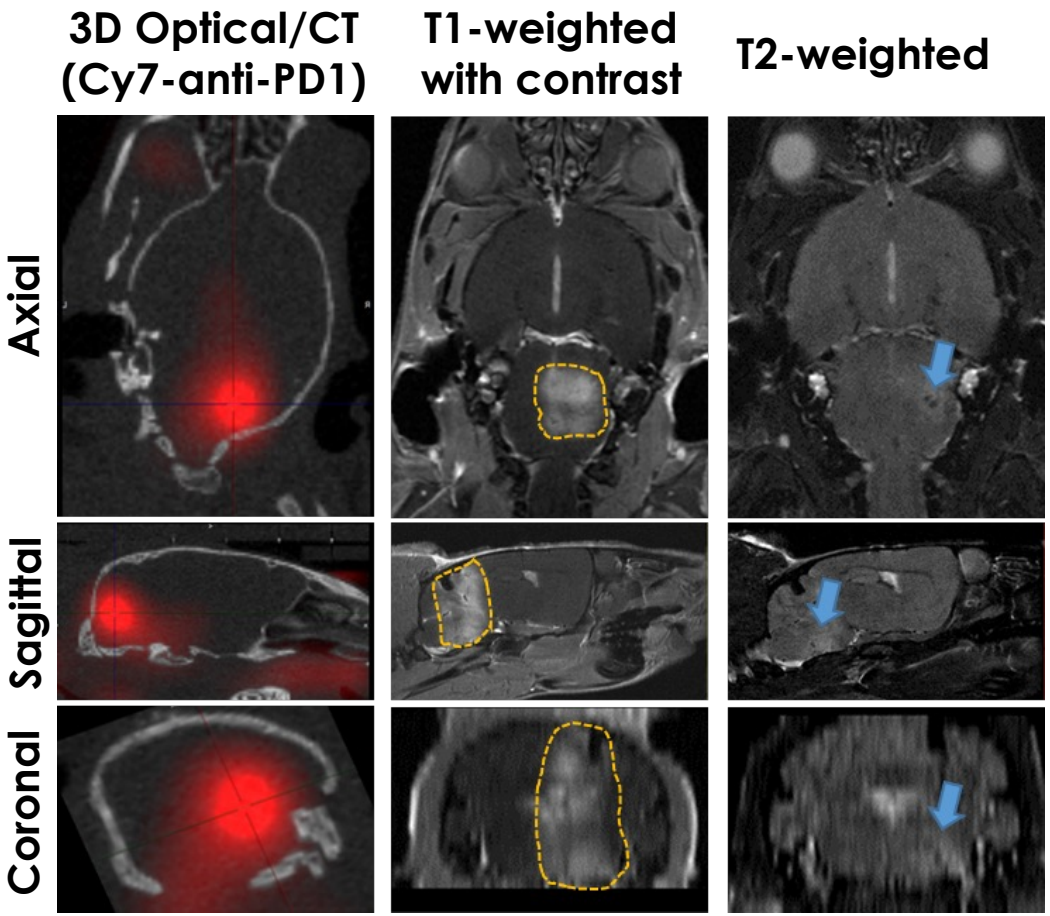
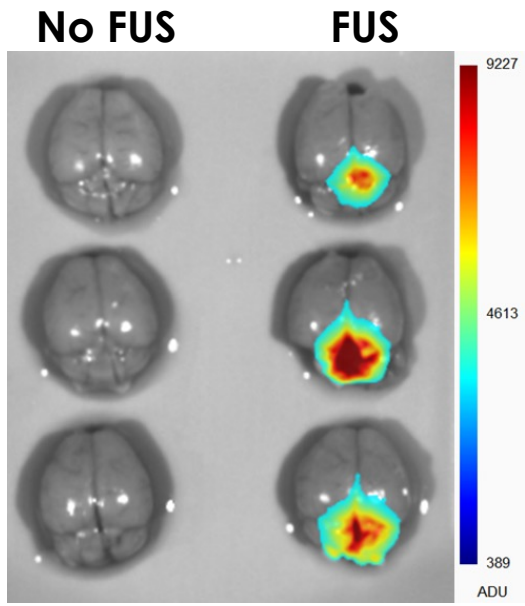
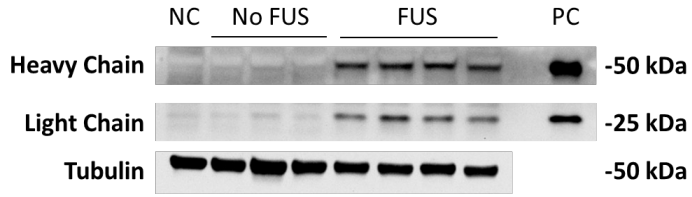
Project 2: FUS Enhances Anti PD1 Delivery







Anti-PD1 antibody is optically labeled.

FUS was delivered to the brainstem

Accumulation of antibody was observed at site of BBB-opening

Ex-vivo imaging showed no optical signal in the brainstem without FUS.

Western blot performed to confirm anti-PD1 delivery

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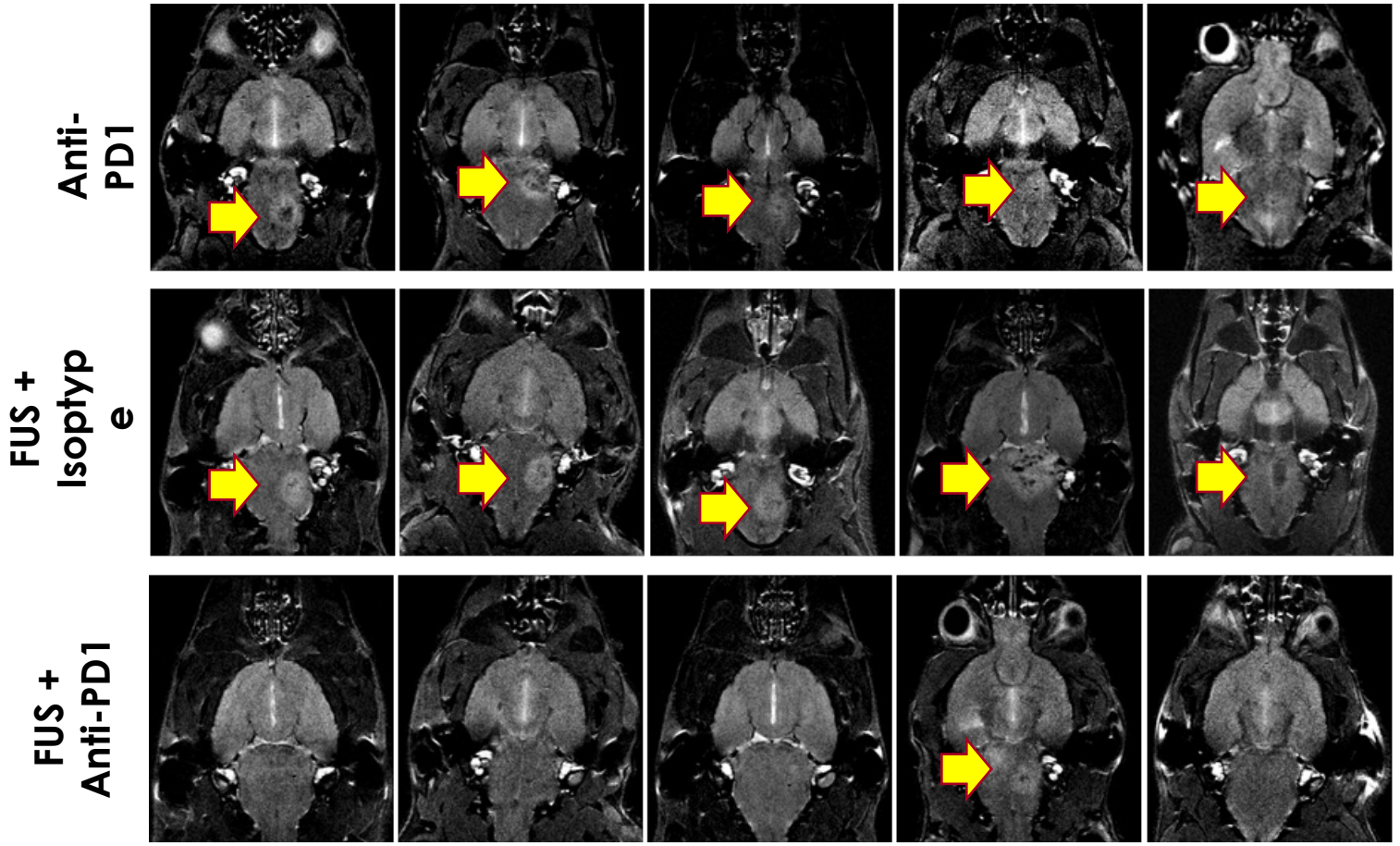
Project 2: FUS and Anti-PD1 Improved Local Control and Overall Survival



MRI imaging showed tumor progression in 5/5 mice treated with either anti-PD1 or FUS+Isotype

Delayed disease progression was seen in FUS+PD1

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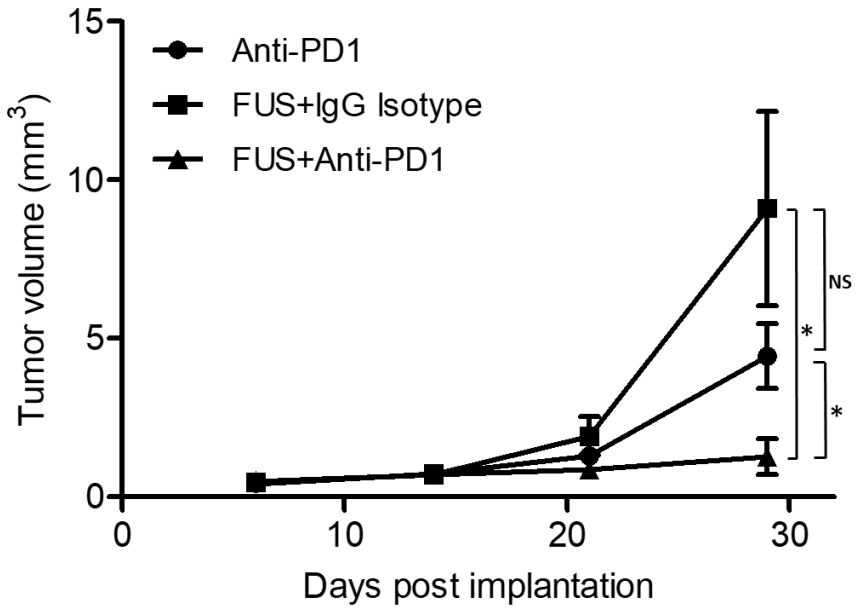
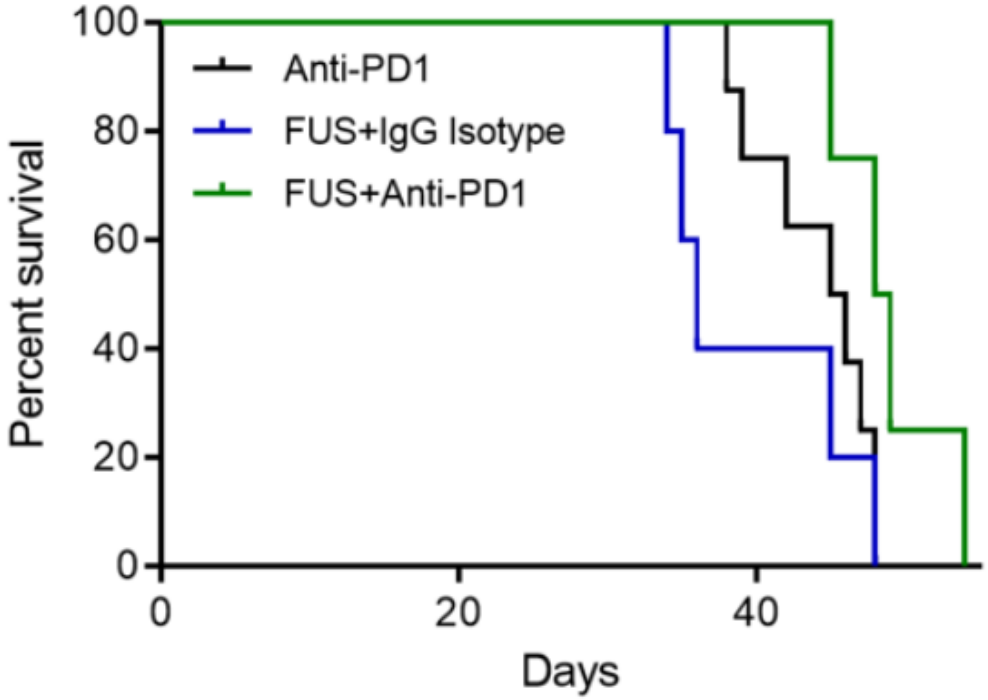
Project 2: FUS+PD1 Improved Local Control and OS





Local control and Overall Survival seen with FUS+PD1

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Thank You!