Development of a Patient-Derived Zebrafish Xenograft Model for Glioblastoma

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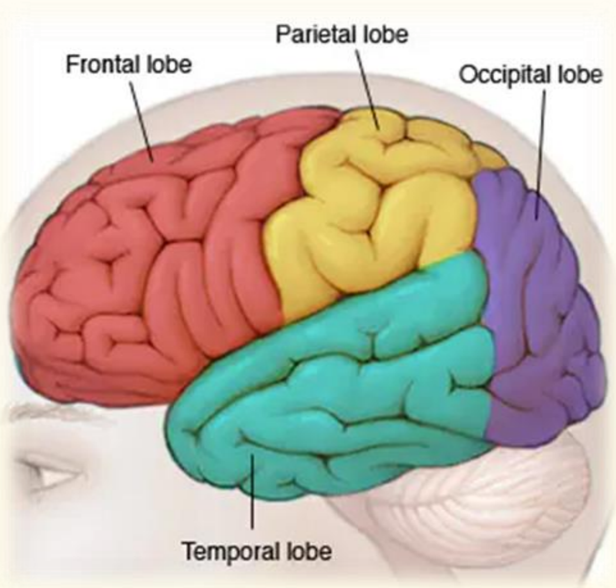
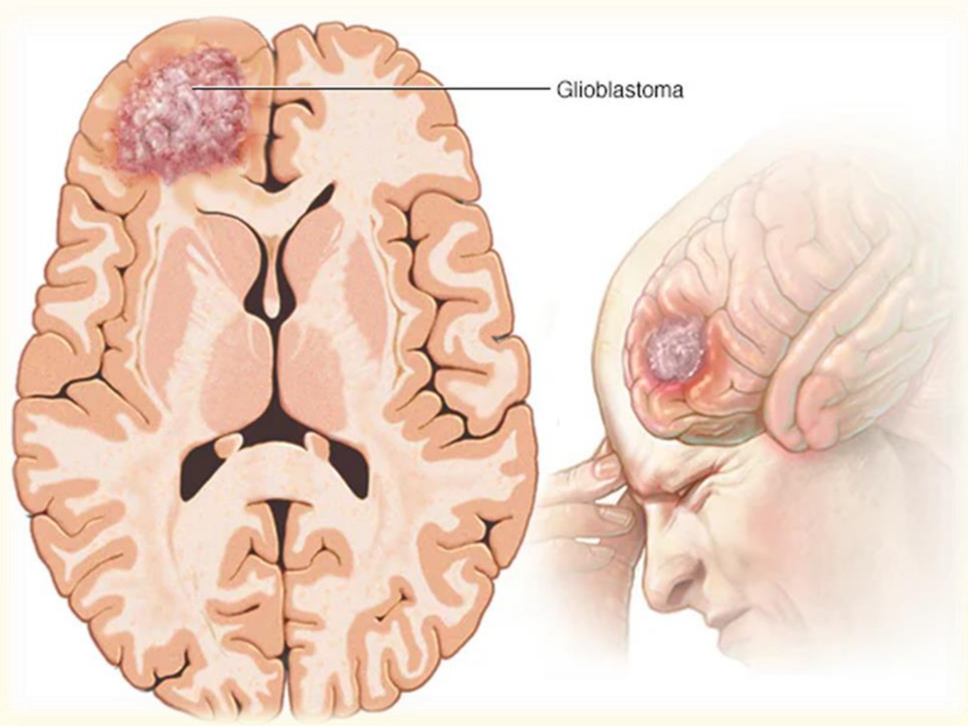
Abstract

Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by rapid proliferation, diffuse infiltration, and resistance to conventional therapies, resulting in poor patient prognosis (Figure 1). Preclinical evaluation of GBM relies heavily on rodent xenograft models; however, these systems are costly, labor-intensive, and often limit high-throughput applications. To address these challenges, we developed a zebrafish xenograft platform as a complementary in vivo model for studying glioblastoma biology. Human glioblastoma cell lines U87 (GFP-labeled) and U251 (mCherry-labeled) were transplanted into zebrafish embryos, enabling real-time visualization of tumor dynamics, including proliferation, migration, and angiogenic interactions within a transparent host environment. This approach further provides a rapid and scalable system for testing therapeutic compounds and assessing tumor responses. By leveraging the advantages of zebrafish—optical accessibility, genetic tractability, and cost-effectiveness—this study establishes a versatile preclinical model that bridges the gap between in vitro assays and mammalian systems, with potential to accelerate glioblastoma research and drug discovery.

Introduction/Background





➢ Symptoms:

- ✓ Seizures
- ✓ Severe headaches
- ✓ Memory and language problems
- ✓ Changes in personality and behavior
- ✓ Muscle weakness or paralysis
- ✓ Loss of sensation or numbness and tingling
- ✓ Fatigue
- ✓ Issues with coordination
- ✓ Speech, hearing, and vision problems

➢ Treatments:

- ✓ Surgery
- ✓ Radiation Therapy
- ✓ Chemotherapy
- ✓ Clinical Trials
- ✓ Tumor Treating Fields (TT Fields)
- ✓ Targeted Therapies

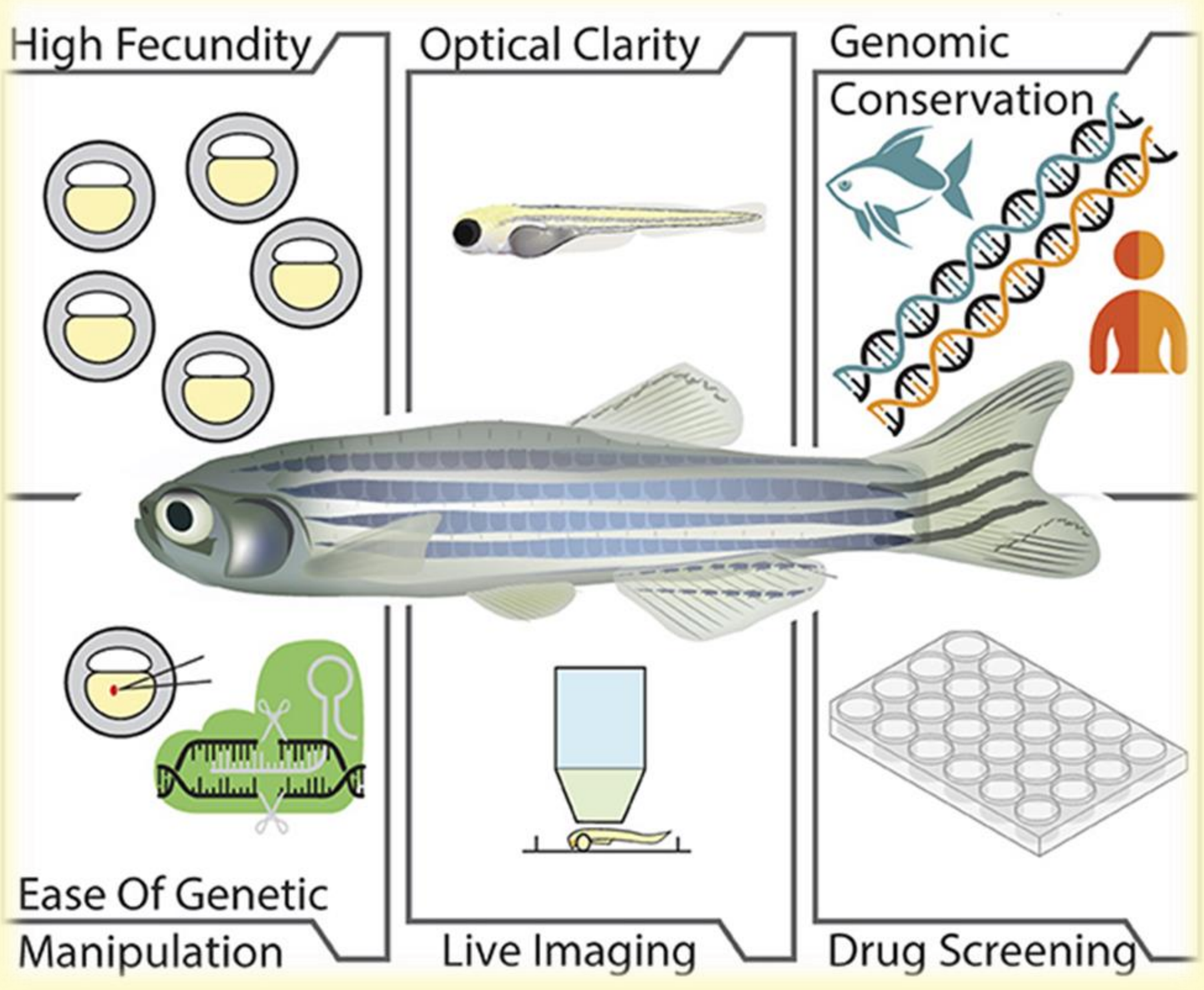
➢ Prognosis: 5-year Relative Survival Rate

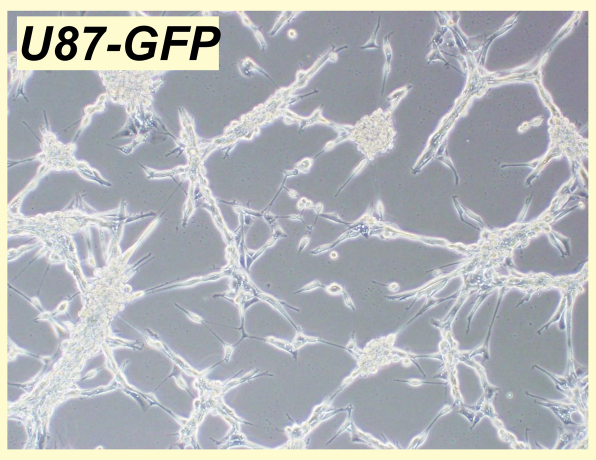
- ✓ Children (ages 0-14): 19.5%
- ✓ Adolescents & Young Adults (ages 15-39): 27.3%
- ✓ Adults (ages 40+): 5.6%

Objectives/Aim

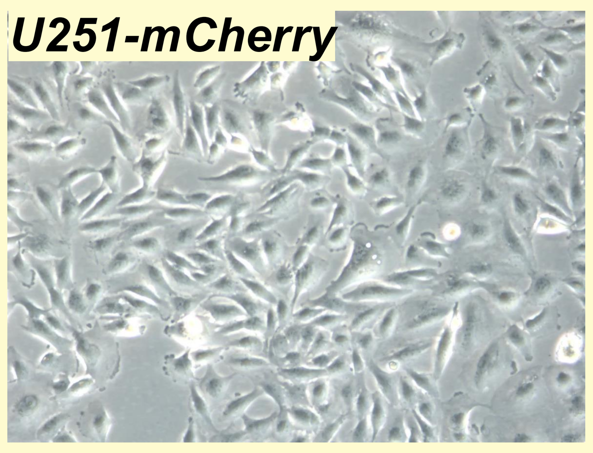
➢ ***To establish a zebrafish glioblastoma xenograft model to understand GBM pathogenesis and treatment options***

Methodology





U87-GFP



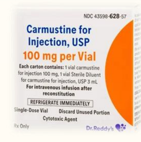
U251-mCherry

Zebrafish Culture

Zebrafish Larvae

Experimental Design

Chemotherapeutic Drugs to Test

Drug	*Mechanism*	*Proposed Dosage Range*	*Administration Method*	*Rationale and Notes*
	*DNA alkylating agent*	*10–100 µM*	*Immersion in E3 medium (0.1% DMSO) Intraperitoneal Injection*	*Standard GBM chemotherapy*
	*Anti-VEGF monoclonal antibody*	*0.1–1 µg/mL*	*Immersion/ Intraperitoneal Injection*	*Large molecule; lower doses due to limited penetration*
	*Alkylating agent*	*5–50 µM*	*Immersion/ Intraperitoneal Injection*	*Crosses blood-brain barrier.*

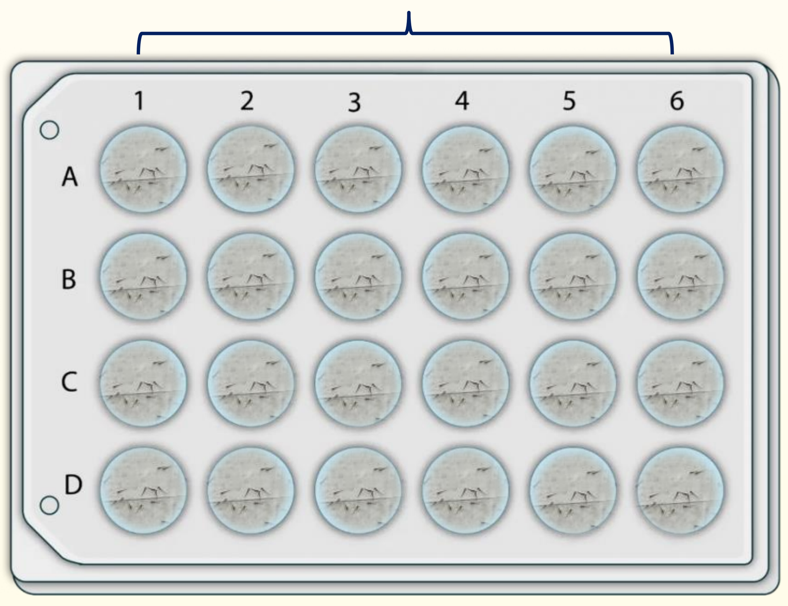
Six Replicates/Treatment

Control - No Injection

Control – Sham Injection

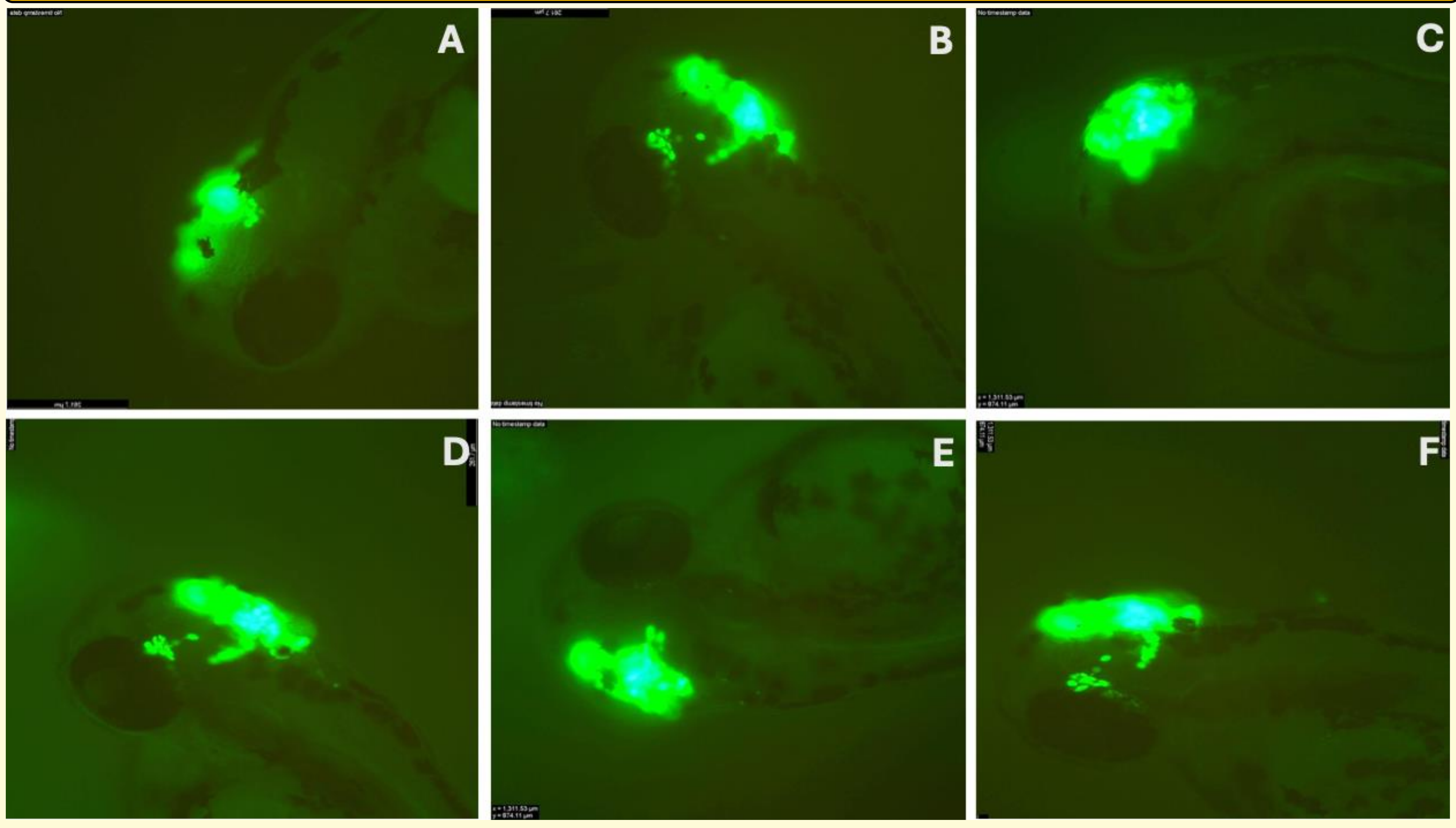
Cancer Injection

Cancer + Drug Immersion

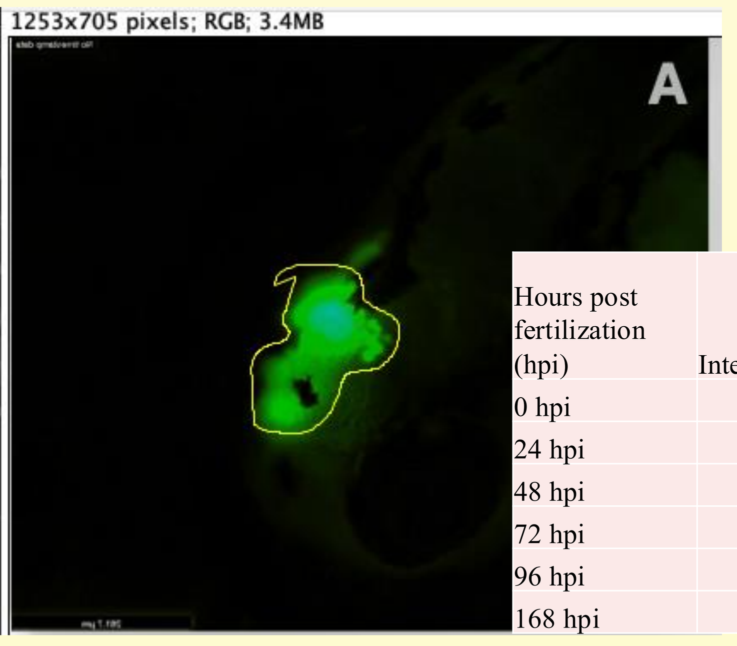


Results

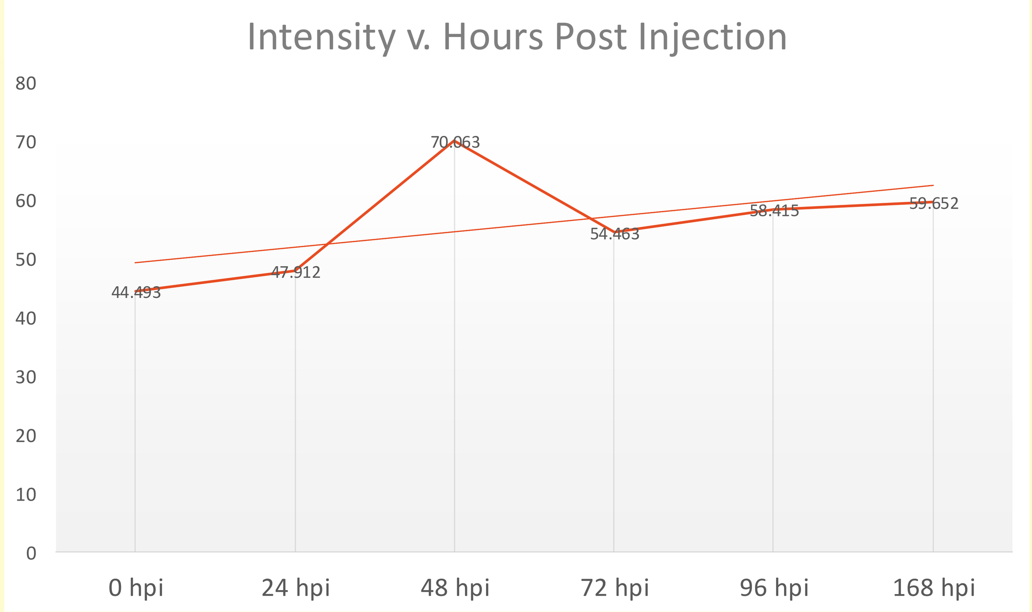
Injection Example



Fluorescence images of zebrafish xenografts injected with glioblastoma cells at 3 days post fertilization. A) 0 hours post injection (hpi), 72 hours post fertilization (hpf); B) 24 hpi, 96 hpf; C) 48 hpi, 120 hpf; D) 72 hpi, 144 hpf; E) 96 hpi, 168 hpf; F) 7 days post injection, 240 hpf.



Hours post fertilization (hpi)	Intensity
0 hpi	44.493
24 hpi	47.912
48 hpi	70.063
72 hpi	54.463
96 hpi	58.415
168 hpi	59.652



Conclusion and Future Studies

Despite low survivability (<10 zebrafish reaching 7 days), the model consistently demonstrated in vivo glioblastoma growth, confirming its feasibility. Optimization of environmental and procedural factors is needed to improve survival. Future studies will test temozolomide, carmustine, and bevacizumab to reduce tumor burden and enhance survivability, refining this model as a reliable, high-throughput platform for preclinical glioblastoma drug testing.

References

- ➢ *Mayo Foundation for Medical Education and Research*
- ➢ *American Brain Tumor Association*
- ➢ *Kesavan, G., Dechorionation of Zebrafish Embryos with Pronase. Oct 18, 2019, protocols.io. https://www.protocols.io/view/dechorionation-of-zebrafish-embryos-with-pronase-f-dm6gp8m2dlzp/v2?step=1*
- ➢ *Doyle, K., Waseem Hasan and Sahar I. Da'as et al., Microinjection quality control in zebrafish model for genetic manipulations. 2021, National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8374492/*

Acknowledgement