AWARD NUMBER: W81XWH-18-1-0291

TITLE: Defining and Targeting the Blood-Brain Barrier in Pediatric Glioma Subgroups

PRINCIPAL INVESTIGATOR: Timothy Phoenix, PhD

CONTRACTING ORGANIZATION: University of Cincinnati
Cincinnati, OH 45221

REPORT DATE: Sept 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2019	Annual	15 Aug 2018 - 14 Aug 2019

4. TITLE AND SUBTITLE
Defining and targeting the blood-brain barrier in pediatric glioma subgroups

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0291

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Timothy Phoenix, PhD

E-Mail: timothy.phoenix@uc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Cincinnati
231 Albert Sabin Way
Medical Sciences Building,
3005M
Cincinnati, OH 45267

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Brain tumors are the leading cause of death from cancers in children. Our recent study demonstrated the presence of BBB heterogeneity within medulloblastoma subgroups, and identified Wnt signaling as a core regulator of brain tumor BBB specification and maintenance. To determine BBB differences in pediatric gliomas we have generated new genetic mouse models of pediatric cortical high-grade (HGG) and brainstem diffuse intrinsic pontine glioma (DIPG) using defined genetic alterations identified in patients. Preliminary data show our DIPG model maintains endothelial Wnt signaling and BBB function, while HGG display heterogeneous BBB disruption, suggesting distinct vascular phenotypes between glioma subgroups. Here we report our progress in characterizing the vasculature of mouse and PDX DIPG models, and features of the mouse models that have led to the development of additional projects. As part of my career development process, I will discuss progress on building my research program and developing collaborations within the field of vascular biology.

15. SUBJECT TERMS
High-grade glioma, blood-brain barrier

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Brain tumors are the leading cause of death from cancers in children. Our recent study demonstrated the presence of BBB heterogeneity within medulloblastoma subgroups, and identified Wnt signaling as a core regulator of brain tumor BBB specification and maintenance. To determine BBB differences in pediatric gliomas we have generated new genetic mouse models of pediatric cortical high-grade (HGG) and brainstem diffuse intrinsic pontine glioma (DIPG) using defined genetic alterations identified in patients. Preliminary data show our DIPG model maintains endothelial Wnt signaling and BBB function, while HGG display heterogeneous BBB disruption, suggesting distinct vascular phenotypes between glioma subgroups. The objective of this proposal is to take an un-biased approach to define BBB function across pediatric glioma subgroups, and to determine if suppression of DIPG Wnt signaling will alter BBB function and improve drug efficacy. Our hypothesis is that regional and/or molecular differences in pediatric glioma subgroups result in vascular BBB heterogeneity, and targeted inhibition of Wnt signaling will increase BBB permeability in DIPG.

2. **KEYWORDS:** High-grade glioma (HGG), Diffuse intrinsic pontine glioma (DIPG), In utero electroporation (IUE), patient derived xenograft (PDX), blood-brain barrier (BBB), endothelium, Wnt signaling.

3. **ACCOMPLISHMENTS:**
   What were the major goals of the project?

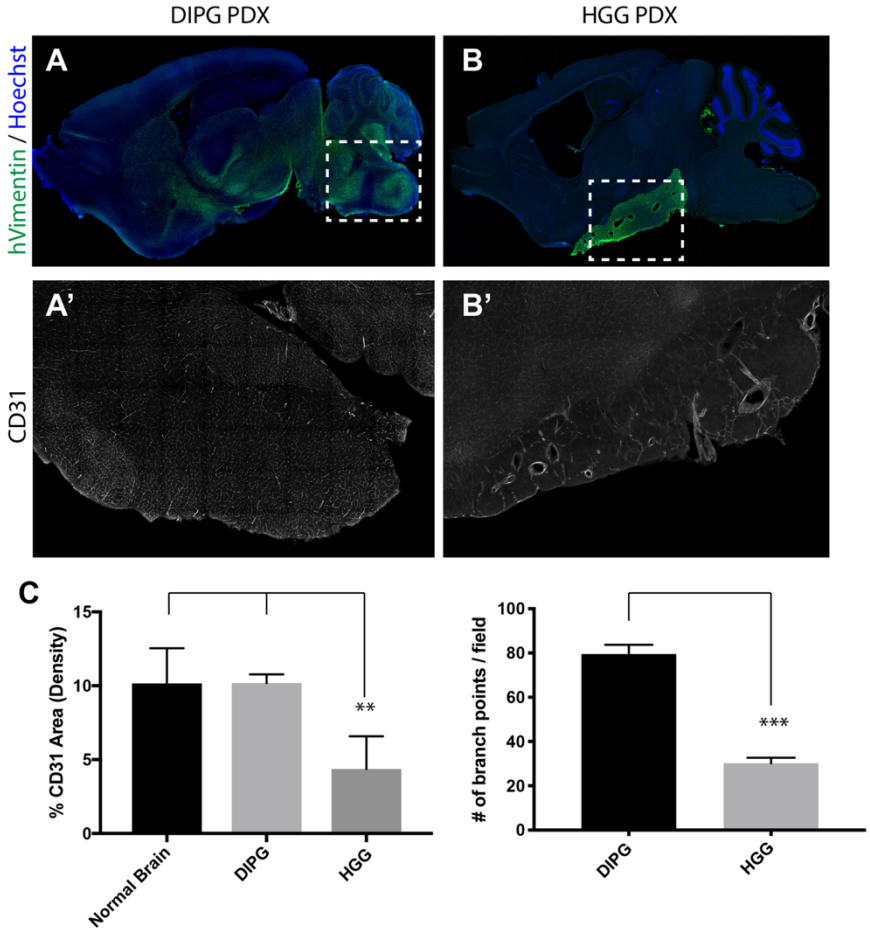
   Below are summaries of each specific task, their projected timeframe from the statement of work, and an update on current results and/or progress.

   What was accomplished under these goals?
   Task 1 – Define vascular BBB properties in pHGG mouse models

   Task 1.1 (estimated timeframe months 1-6) – characterize blood vessel phenotypes in patient derived samples. PDX samples orthotopically implanted in the brains of CD1-nude mice will be provided by our collaborator, Dr. Hulleman, VUMC). PDX samples will include cortical HGG and DIPG/DMG samples.

   This task is partially completed, and still in the analysis phase. We have received a series of PDX implanted brains from Dr. Hulleman. These include cortical HGG/GBM samples (VUMC-05, harbors BRAF V600E mutation), DIPG (HSJD-07 harbors H3.1 K27M mutation, VUMC DIPG-F, H3.3K27M mutation) and a thalamic DMG (VUMC-X, H3.3K27M mutation). One of the major challenges we have faced is the condition of the brains sent to us. The brains were collected as we asked for (4% PFA perfusion, sucrose protected), but were not frozen following cryoprotection, and were stored at 4 degrees until all samples were ready to ship. We have embedded all samples, sectioned, and stained them for our panel of vascular and BBB antibodies. The storage conditions prior to shipment seem to have affected the epitopes, as many samples have high background and weak staining. But we have successfully been able to work-up stains for a number of antibodies, including CD31 (pan-endothelial marker), Glut1 (endothelial BBB), and Cldn5 (endothelial tight junction). The cortical HGG/GBM PDX model is characterized by forming larger, dense cortical

tumors. Tumors contain sparse blood vessels that are enlarged and abnormal at the morphological level. Glut1, which is normally expressed by CNS endothelial cells, is highly expressed in the tumor cells, and either absent or weakly present in cortical tumor endothelium. Unfortunately we haven't gotten the BBB permeable marker Plvap to work on the PDX samples yet (determined by negative staining on internal control tissue – choroid plexus; we have run additional non-pdx samples as positive controls to verify antibody works). Compared to cortical HGG/GBM samples, all DIPG PDX samples have displayed vasculature that is indistinguishable from normal brain. DIPG PDX samples show a range of cellular density, with some samples more diffuse, and others showing a higher density of tumor cells within the brainstem area (visualized by staining for human vimentin). Glut1 staining is restricted to the endothelium in all DIPG samples, and the tight junction marker Cldn5 displays consistent organized structures within the vessels. Finally, we have also begun analyzing thalamic DMG PDX samples, tumor cells can be found all over the brain, including brainstem, cerebellum, and cortex. Cellular density is fairly high (seen by Hoechst staining). Similar to DIPG PDX samples, the thalamic DMG tumors display normal vessel architecture by CD31



staining, and Glut1 staining is only present in blood vessels. Thus, it appears that H3 K27M mutant DIPG and DMG PDX models maintain vascular architecture and BBB integrity significantly better than cortical HGG/GBM PDX models. (FIG 1).

Figure 1 – Preliminary analysis of vasculature in DIPG and HGG PDX samples. (**A**) Representative tile scanning image of DIPG PDX sample stained for hVimentin (green) to identify tumor cells, and Hochest to identify all nuclei. DIPG PDX tumor cells could be seen infiltrating though out the brainstem and into different brain regions (cerebellum, cortical hemisphere). Hoechst staining confirmed increased cellular density in regions with tumor cells.
(**A'**) Tiled image of CD31 staining labeling all blood vessels in the GFP-positive brainstem region of a DIPG PDX sample. (**B**) Representative tile scanning image of HGG PDX sample stained for hVimentin (green) to identify tumor cells, and Hochest to identify all nuclei. HGG PDX tumors were usually seen growing in a more defined pattern, either within the cortical hemisphere, or in adjacent space. Tumor cells identified by hVimentin staining displayed limited infiltration, usually limited to nearby the primary tumor site. (**B'**) Tiled image of CD31 staining labeling all blood vessels in the GFP-positive HGG tumor region and adjacent cortical region. Note the enlarged vessels throughout HGG tumor region. (**C**) Quantification of CD31-positive stained vessels in normal brain (both cortex and brainstem regions), GFP-positive DIPG regions, and GFP-positive HGG regions. Note a significant decrease in the overall density of vessels within HGG tumors. (**D**) Quantification of branch points per field in DIPG and HGG PDX samples. HGG vessels showed a decrease in branching.

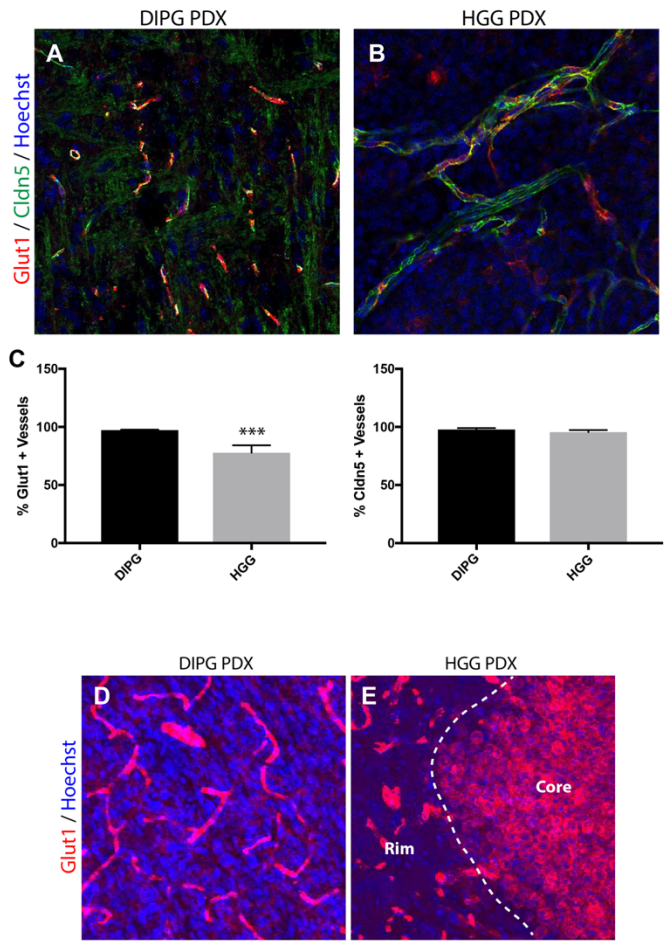


Figure 2 – Preliminary analysis of BBB associated markers in DIPG and HGG PDX samples. (**A,B**) Representative images of Glut1 (red) and Cldn5 (green) stained sections of DIPG and HGG PDX samples. DIPG PDX samples displayed strong Glut1 staining in blood vessels both within and outside of tumor areas. Cldn5 staining for tight junctions displayed organized patterns within vessels. (**C**) Quantification of vascular Glut1 indicates a significant decrease in Glut1 vascular staining in HGG samples. Although it is not totally gone in HGG, and is still present in the majority of vessels. Quantification of Cldn5 staining did not show expression level changes between DIPG and HGG PDX samples. We do note differences in the overall organizational pattern of Cldn5, with HGG tumor Cldn5 junctions appearing more disorganized compared to DIPG and control brain regions. (**D,E**). Additional images of DIPG and HGG PDX samples stained with Glut1 and Hoechst. Even in higher tumor density regions, Glut1 staining remained restricted to vessels in DIPG PDX samples. In HGG PDX samples, Glut1 stained switched to tumor cells in certain "core" regions, and could be found in blood vessels in adjacent regions that contained a mixture of infiltrating tumor cells and normal brain.

Subtask 1.2: (estimated timeframe 1-4 months) - Generate Piggybac DNA plasmids that express PDGFB, PDGFRA, DNp53, and H3.3 WT, K27M, and G34R under the CAG promoter.

We have completed the construction for all of these plasmids. All were generated by PCR amplification and cloning into PB-CAG-Ires-GFP/Luciferase plasmids using the InFusion kit (Clontech). All plasmids were verified by sanger sequencing at the CCHMC DNA sequencing core.

Subtask 1.3: (estimated timeframe 4-12 months) – Mouse models: electroporate DNA constructs produced in task 1.2 into embryonic mouse cortex or brainstem and monitor survival of mice. Groups will include cortical and brainstem controls (H3.3WT + DNp53), cortical HGG (H3.3 G34R + DNp53 + PdgfraD842V) and brainstem DIPG (H3.3 K27M + DNp53 + PdgfraD842V).

We have completed of this subtask. We have created control cortex, control brainstem, cortical HGG, and brainstem DIPG models. Pups positive for bioluminescent signal were monitored for development of symptoms related to brain tumor burden. DIPG models display the shortest latency to tumor development, developing head tilts and neurological gate problems before euthanasia (median survival = 30 days postnatal). Cortical HGG models displayed a longer latency (median survival = 90 days postnatal), and were prone to display domed heads and general lethargy as symptoms. Cortical HGG models displayed rapid deterioration after initial symptom presentation which required euthanasia. This rapid progression was not commonly seen in DIPG models. We hypothesize that this is related to intratumoral hemorrhaging, which we have seen before in other mouse brain tumor models cause a rapid decline in health The vast majority of control cortex and brainstem mice were symptom and tumor free, although we did find one brainstem, and three cortical samples that appeared to develop tumors. It was interesting to note that compared to previous empty vector control samples, overexpression of H3.3WT resulted in an eventual reduction in GFP-positive cells. We also noted a decreased GFP signal in H3.3WT control brains (both brainstem and cortex), and also in decrease or a loss of bioluminescent signal as mice got older. Overexpression of H3.3WT may result in some cellular toxicity or stress, leading to fewer cells present, and an increased risk of spontaneous tumor development. TMR-dextran injections were performed on a subset of mice in each group. All control brainstem, control cortex, and DIPG model samples did not show extravascular leakage of red TMR-dextran dye. HGG model samples regularly displayed diffuse TMR-dextran signal with the GFP-positive tumor region, indicated extravascular leakage. Ongoing analysis of sections from these TMR-dextran injected brains is ongoing. Any additional tumor bearing mice are not part of this award.

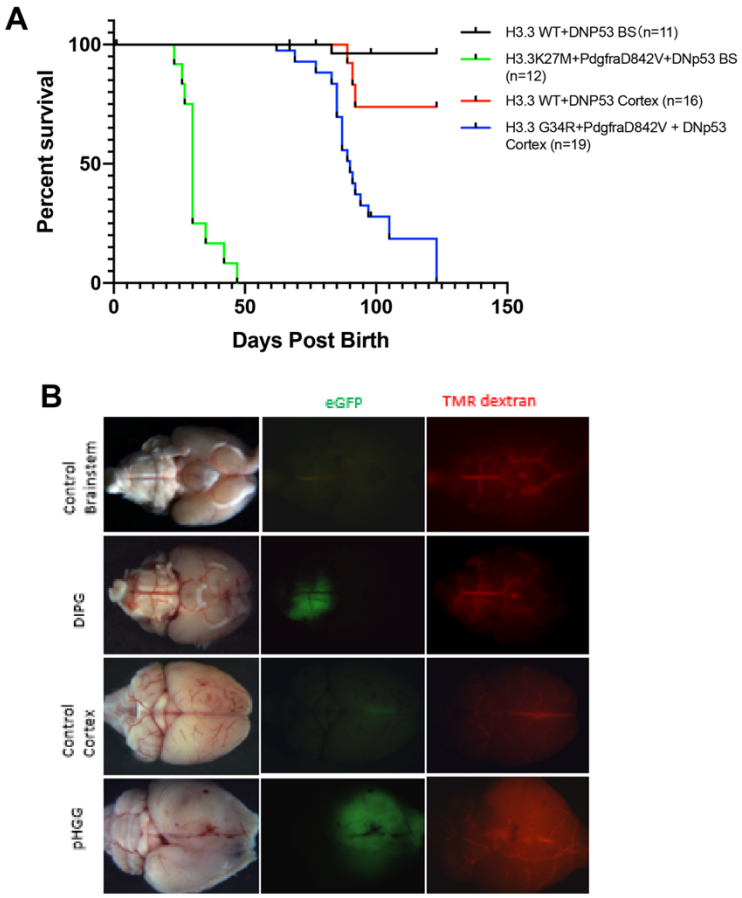


Figure 3 – Survival curves for controls (brainstem and cortex), DIPG, and HGG mouse models created by in utero electroporation. (**A**) Survival curve for control brainstem (H3.3WT + Dnp53), control cortex (H3.3WT + DNp53), DIPG (H3.3K27M + PdgfraD842V + DNp53), and HGG (H3.3G34R + PdgfraD842V + DNp53) mouse models. (**B**) Representative whole brain images of control brainstem, DIPG, control cortex, and HGG mouse models injected with TMR-dextran. Note the diffuse red TMR-dextran signal present in the HGG sample.

Subtask 1.4: (estimated timeframe 6-12 months) – Immuno-stain and quantification of mouse tumors produced in task 1.3

Most of this task has been completed, or is in the process of being analyzed. We have collected brains from the above task 1.3, and processed them for immunostaining with our antibody panels. We also injected a subset of mice from each group with 10kDa TMR-dextran, providing a method to measure extravascular tracer leakage. We have stained sections with CD31, and measured vascular density (% area of CD31 stain), vascular branch points, and vessel diameter.

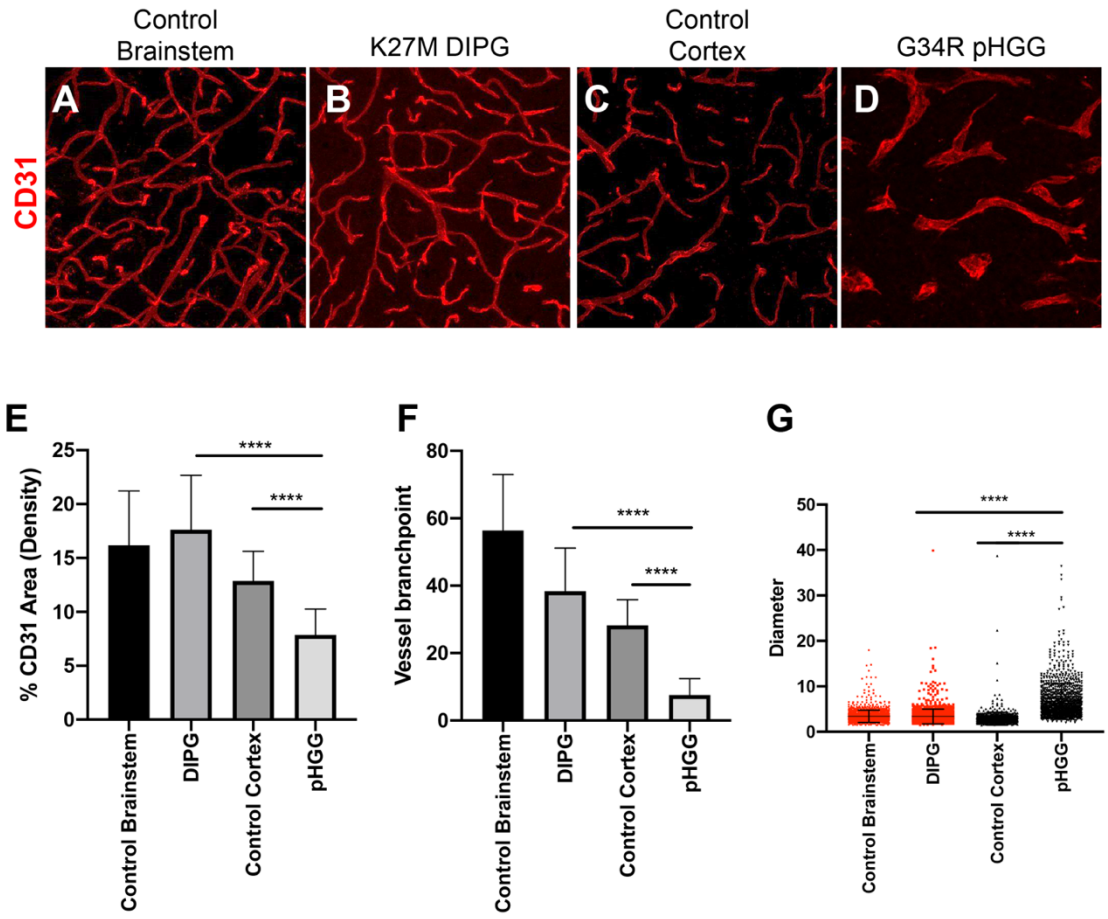


Figure 4 – Cortical HGG mouse models display vascular abnormalities. (A-D) Representative images of control brainstem, K27M DIPG tumors, control cortex, and G34R cortical HGG tumor sections stained for CD31 (red). Note the organized and consistent pattern of blood vessels in control and DIPG tumors. Cortical HGG consistently displayed enlarged and abnormal blood vessels within the tumors. (E) Quantification of CD31 staining. (F) Quantification of vessel branch points per field. HGG tumors display a lower overall density of blood vessels, and a decrease in vessel branching. (G) Quantification of vessel diameter. HGG tumors display consistently enlarged blood vessels compared to DIPG and control cortex tissue.

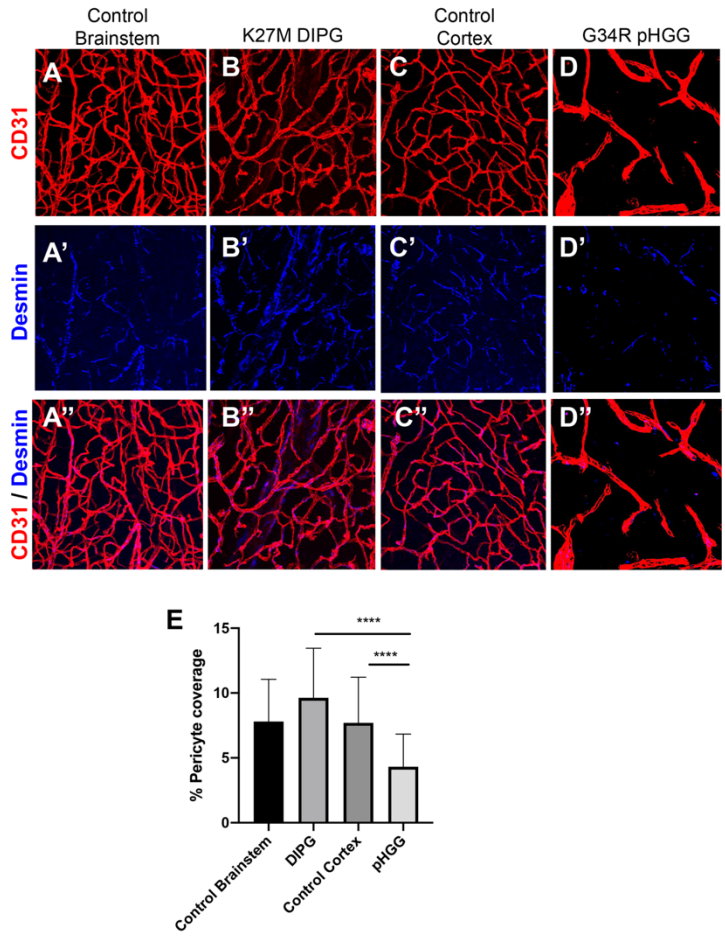


Figure 5 – Decreased pericyte coverage in HGG mouse models. (A-A'') Individual and merged images for control brainstem stained for CD31 (red) and the pericyte marker desmin (blue). (B-B'') Individual and merged images for DIPG tumors stained for CD31 (red) and the pericyte marker desmin (blue). (C-C'') Individual and merged images for control cortex stained for CD31 (red) and the pericyte marker desmin (blue). (D-D'') Individual and merged images for HGG stained for CD31 (red) and the pericyte marker desmin (blue). (E) Quantification of pericyte coverage (% area of desmin staining within CD31+ regions). HGG tumors display a constant decrease in pericyte endothelial coverage. No differences were noted in DIPG tumor models.

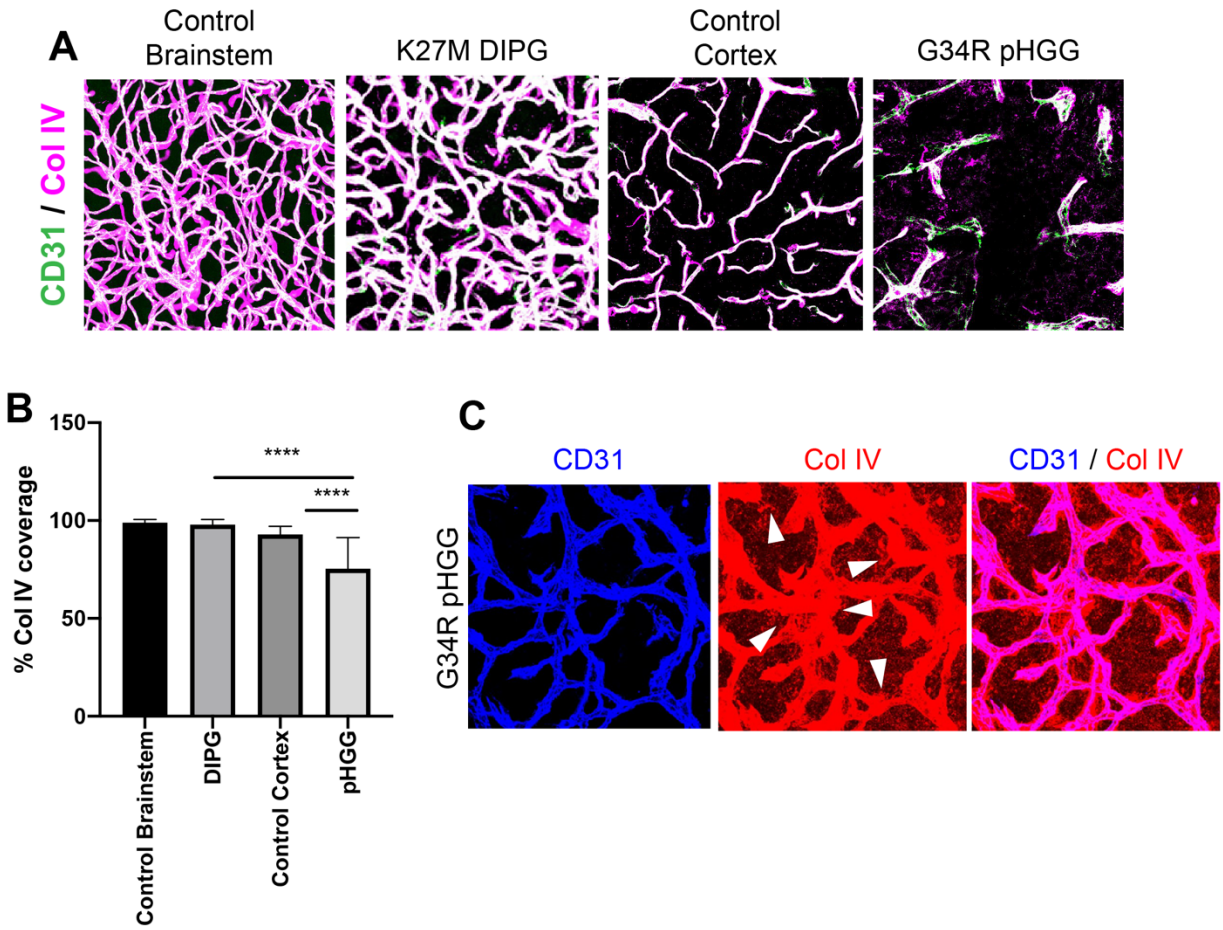


Figure 6 – Decreased and altered extracellular matrix coverage in HGG mouse models. (**A**) images of sections from each condition stained for CD31 (green) and Collagen IV (magenta). (**B**) Quantification of Col IV vascular coverage across conditions. Note a decrease in the Col IV endothelial coverage in HGG tumors. (**C**) Higher magnification images of a HGG tumor stained with CD31 (blue) and Col IV (red). ECM fragment are commonly found in HGG tumors, which looks like they are being chewed up / degraded around vessels. This is not seen with any regular frequency in DIPG tumor models.

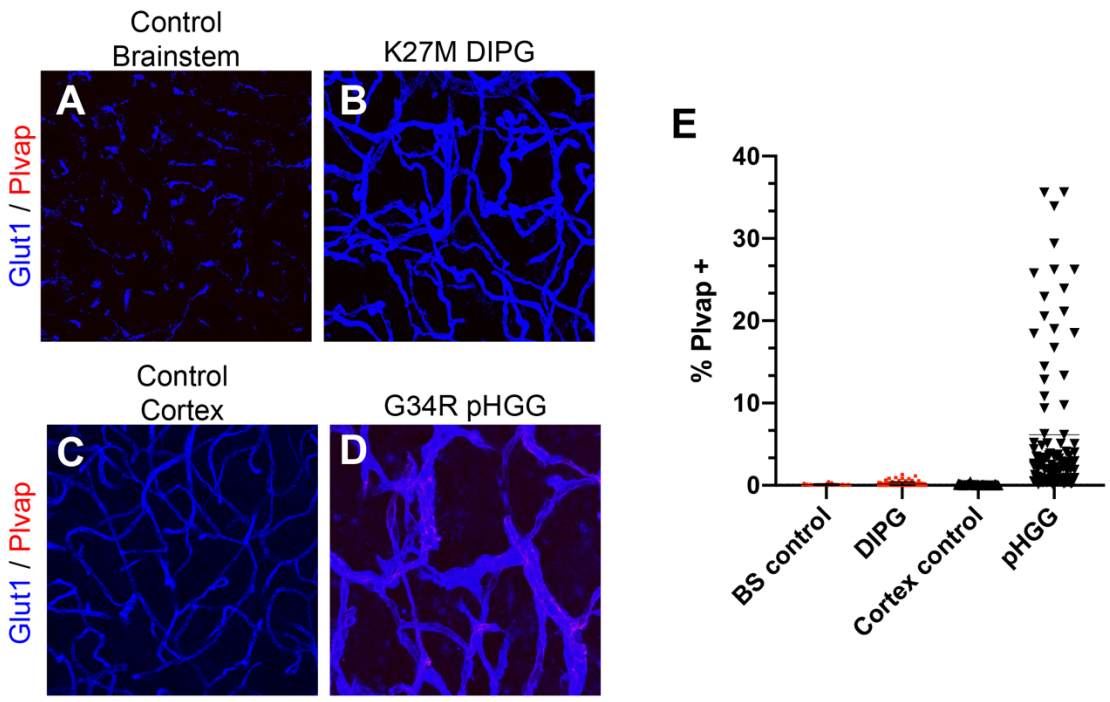


Figure 7 – Increased Plvap expression in HGG mouse models. (A-D) Images of control and tumor sections stained for Glut1 (blue) and Plvap (red). (E) Quantification of Plvap+ stained vessels across conditions. We do not see Plvap expression in control brainstem, control cortex, or DIPG tumors. HGG tumors blood vessels are mostly Glut1-positive, but do display regions of Plvap-positivity. Maintenance of Glut1 expression, but increases in BBB permeability may point to pericyte dysfunction as the initial cause of vessel changes, which may lead to more severe Wnt deregulation.

Task 2: molecular profile of mouse tumor endothelial cells.

Subtask 2.1: (estimated timeframe 10-14 months) – Generate additional mouse model tumors as done in task 1.3.

We have generated the majority of these mice, and the remaining few are ongoing. This includes control cortex, control brainstem, cortical HGG, and brainstem DIPG.

Subtask 2.2: (estimated timeframe 12-24 months) – Identify molecular differences in tumor endothelial cells using RNA sequencing. Mouse models samples generated in task 2.1. CD31+/CD45- endothelial purification, RNA isolation, and RNA sequencing.

We have been performing endothelial purifications, and optimizing our protocol for these tissue and diffuse tumors that contain large amounts of myelin debris. We have been using CD45-/CD31+ selection by magnetic bead sorting, as scheduling sorting time at the CCHMC core that matches when tumors develop has proven difficult. We have been performing test isolations from mice not associated with this study, to optimize the amount of cells purified, total RNA, and using qPCR to validate the selection of cell types based on marker expression. We have seen good endothelial gene enrichment (Cd31, Tie2, Vegfr2) and decreased neuronal (NeuN, Tub3) and microglia (Cd68) in our CD45-/CD31+ isolations, indicating our samples are highly enriched for endothelial cells. We do see enrichment for pericyte markers (Pdgfrb, Abbc9) in our CD31+/CD45- sorted cells, suggesting there is some level of pericyte contamination in the endothelial purified population. This is very commonly noted in the literature too. We can either include an additional sorting step (Pdgfrb-negative selection), and re-purify, or continue on with the noted pericyte levels. Since pericyte dysfunction is also thought to contribute to pathogenic processes, it may add an additional layer onto our dataset.

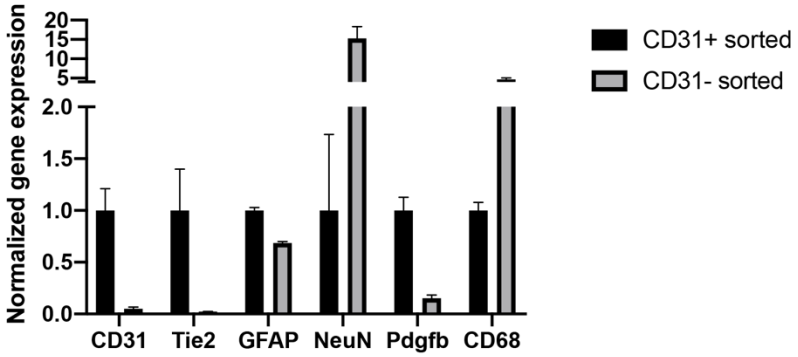


Figure 10 – Validation of endothelial purification from control brainstem samples. We have isolated CD31+/CD45- fractions, and CD31- fractions from normal brainstem. Total RNA was isolated, and cDNA synthesized. qPCR reactions were performed in triplicate with endothelial, astrocyte, neuron, pericyte, and microglia/macrophage markers.

Outside of the proposed study here, we are now planning to perform single-cell RNA sequencing on endothelial cells from at minimum control brainstem and brainstem DIPG samples. We think this will provide added resolution, and resolve any problems that may be encountered with cell contamination (pericyte, etc) during purification.

Major Task 3: Wnt inhibition in DIPG mouse model

Subtask 3.1: (estimated timeframe 20-24 months) – Generate Piggybac DNA plasmids that express Dkk1 and Fz8-CRD-IgG, or Crispr/Cas9 plasmids targeting Porcn, Wnt7a, Wnt7b, and Ndp to inhibit Wnt signaling.

We have made Dkk1 and Fzd8-CRD-IgG overexpression constructs. They are verified by sanger sequencing, and we have transfected them into 293 cells and verified expression at the transcript level (qPCR). We have initiated CRISPR-Cas9 work outside of this study, testing the efficiency and detection methods in our IUE mouse models. We have found it works quite well with guides targeting genes of interest in other projects (Ppm1d, Angpt1), and have worked out both NGS and sanger sequencing methods to detect cutting and INDEL formation rates (data not shown for Ppm1d and Angpt1, not part of this award). We will begin generating gRNAs targeting Wnt related genes soon.

Subtask 3.2: (estimated timeframe 24-30 months) – Co-electroporate expression (Dkk1 or Fz8-CRD-IgG) or Crispr/Cas9 (Porcn, Wnt7a, Wnt7b, and Ndp) DNA constructs with those in task 1.2 to produce tumors lacking Wnt signaling.

This work has not been initiated yet.

Subtask 3.3: (estimated timeframe 30-36 months) – Immunolabel and quantification of mouse tumors produced in task 3.2.

This work has not been initiated yet.

Subtask 3.4: (estimated timeframe 30-36 months) – Efficacy and pharmacodynamics studies of Ribociclib on control and Wnt inhibited tumors.

This work has not been initiated yet.

What opportunities for training and professional development has the project provided?

(1) Attended and presented a poster on our project at the 2019 Cold Spring Harbor Blood-brain barrier meeting. This was an outstanding meeting, and I learned a lot of new things about the BBB in the normal and diseased setting, along with new techniques being used to study them. Highlights included (**A**) regional BBB difference work by Richard Daneman's group. I spoke with them about comparing datasets once ours is generated. The Daneman group was also looking at BBB dysfunction in pathological (non-tumor) settings. They consistently found decreased endothelial Wnt signaling across different diseases (stroke, demyelination, etc), which is reassuring. (**B**) I was introduced to Benoit VanHollebeke from Belgium. He's one of the new co-chairs for the 2021 CSH BBB meeting. His lab has developed a new method to activate endothelial specific Wnt signaling, and wanted to form a collaboration study it in brain tumors after learning about our work. We have discussed this a few times post-meeting, and are setting it up as both a short and long-term collaborative study.

(2) I continue to attend regular meetings at CCHMC to present our work and get feedback. Meetings with Dr. Ratner provide guidance on career development and moving onto the next steps.

(3) I submitted a grant application on a developing project that is based off of our IUE DIPG/HGG mouse model work. We have identified Angpt1 as a potential modulator of DIPG BBB integrity.

This grant was funded by the Pediatric Brain Tumor Foundation (Young Investigator Award; Awarded 9/15/2019).

(4) We submitted a manuscript characterizing our brainstem IUE DIPG mouse models to Neuro-Oncology. After revisions, the manuscript is provisionally accepted pending minor edits.

How were the results disseminated to communities of interest?

Cold Spring Harbor, 2019 Blood-brain barrier meeting. Invited poster presentation.

What do you plan to do during the next reporting period to accomplish the goals?
We plan to complete analyses remaining in Task1, submit and analyze RNA samples in Task 2, and being working on Task 3.

4. IMPACT:

1. We have initiated vascular BBB analyses in pediatric HGG and DIPG samples – in both PDX and DIPG mouse models.
2. DIPG samples from both PDX and our mouse models consistently display limited to no vascular abnormalities, and maintenance of BBB integrity.
3. HGG tumors display a more classic tumor core – with disrupted BBB integrity, and a more intact BBB in the rim region. We note that our HGG mouse models show a very nice rim region that can be fairly large. PDX HGG samples to date tend to have a smaller infiltrative edge / rim, but they are present.
4. We have identified Angpt1-Tie2 signaling as a potential mechanism that promotes the maintenance of BBB integrity. This work was recently funded by the Pediatric Brain Tumor Foundation. We hypothesize that Angpt1-Tie2 signaling actively promotes BBB integrity in DIPG/HGG, and that inhibition results in BBB dysfunction and increased angiogenesis.
5. We have generated a number of tumor cell lines from our DIPG and HGG mouse models.
6. Building new research collaborations to expand our knowledge of tumor-vascular interactions.

What was the impact on other disciplines?
"Nothing to Report."

What was the impact on technology transfer?
"Nothing to Report."

What was the impact on society beyond science and technology?
"Nothing to Report."

5. **CHANGES/PROBLEMS** *"Nothing to Report."*
Changes in approach and reasons for change
"Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
"Nothing to Report."

Changes that had a significant impact on expenditures
"Nothing to Report."

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
"Nothing to Report."

Significant changes in use or care of human subjects
"Nothing to Report."

Significant changes in use or care of vertebrate animals
"Nothing to Report."

Significant changes in use of biohazards and/or select agents
"Nothing to Report."

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  - *"Nothing to Report."*

Journal publications. *"Nothing to Report."*

Books or other non-periodical, one-time publications. *"Nothing to Report."*

Other publications, conference papers and presentations.

Cold Spring Harbor, 2019 Blood-brain barrier meeting. Invited poster presentation.

- **Website(s) or other Internet site(s)**

"Nothing to Report."

- **Technologies or techniques**
  - *"Nothing to Report."*

- **Inventions, patent applications, and/or licenses**
  - *"Nothing to Report."*

- **Other Products**

"Nothing to Report."

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
No changes:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Active Research Support
Pediatric Brain Tumor Foundation - Early Career Development Award. 9/15/19 – 9/14/22
Title: Maintenance of DIPG blood-brain barrier integrity by Angiopoietin1
Role: Phoenix (PI) – 20% effort
This study aims to determine the regulation and function of Angpt1 in regulating DIPG blood-brain barrier integrity. We will define the expression pattern of Angpt1 in DIPG mouse models, and determine how Angpt1 deletion influences BBB function. We will also investigate how Angpt1 loss impacts the endothelial gene exp.

IronMatt Research Grant - Mathew Larson Foundation 6/1/2019 – 5/31/2020
Title: Targeting immune evasion in diffuse intrinsic pontine glioma
Role: Phoenix (PI) – 10% effort
This objective of this study is to examine tumor response and immune cell infiltration into spontaneous DIPG mouse models treated with CDK4/6 inhibitors, immune-check point inhibitors, or a combination of both. We will also perform *in vitro* screening of other potential immune modulatory drugs recently identified using primary tumor cell lines generated from our murine DIPG models.

Project # CA171185 10/15/18 – 9/30/21
Department of Defense PRCRP Career Development Award
Defining and Targeting Therapeutic Barriers in Pediatric Glioma Subgroups
Role: Phoenix (PI) – 30% effort
This study aims to examine the vascular phenotype and blood-brain barrier (BBB) function in newly developed mouse models of supratentorial pediatric high-grade glioma and diffuse intrinsic

pontine glioma. We will define subgroup vascular differences, and investigate the role of Wnt signaling in BBB integrity.

Completed Research Support (within past 3-years)
Project # NCATS _ KL2 TR001426 (PI, Phoenix) 4/1/17 – 3/1/19
CCTST CT2 Mentored Career Development Award Research expenses only
Defining and Targeting Therapeutic Barriers in Brain Tumors

Project # no number assigned (PI: Phoenix) 12/1/16 – 11/20/17
UC Brain Tumor Center MTP Research expenses only
Determining Mechanisms of Metastasis in Medulloblastoma Subgroups

Project # no number assigned (PI: Patel, Co-PI: Phoenix) 1/1/17 – 6/30/17
UC Gardner Neuroscience Institute Pilot Program Research expenses only
Developing Novel Mouse Models of Pediatric Brain Tumors

What other organizations were involved as partners?
"Nothing to Report."

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*