Form Approved Through 6/30/2012 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.		
Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Preclinical evaluation of systemic and direct delivery of a PDGFR-alpha antibody

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☒ NO ☐ YES
(If "Yes," state number and title)
Number: Title:

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. eRA Commons User Name
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3c. POSITION TITLE
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Pediatrics

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School of Medicine

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4. HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
☒ No ☐ Yes	☐ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
	☒ No ☐ Yes	☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☒ Yes — 5a. Animal Welfare Assurance No A3195-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
09/01/11	08/31/12	$82,049	$82,049	$82,049	$82,049

9. APPLICANT ORGANIZATION
Name Duke University
Address
2200 West Main St.
Suite 820 Erwin Square Plaza
Durham, NC 27705

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☒ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
56-0532129
DUNS NO 044387793 Cong. District 04

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13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
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14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* Laurie A. Henry — DATE 05/27/11

Oren Becher — 5/31/11
Oren Becher, M.D. Date

Section 2.

Title: Preclinical evaluation of systemic and direct delivery of a PDGFR-alpha neutralizing antibody in a brainstem glioma mouse model

Hypothesis- Genetically engineered mouse models of cancer are useful to elucidate mechanisms of tumorigenesis, and can serve as preclinical models for the evaluation of novel agents. Rare tumors such as brainstem gliomas (BSGs) require genetically accurate preclinical models, which recapitulate the genetic alterations seen in the human disease, as preclinical tools. Because there are increasing numbers of available novel therapeutics, there is a need to prioritize the best combinations to translate into clinical trials. Although there have been numerous clinical trials evaluating novel agents to treat BSGs, **none of them has been shown to significantly affect prognosis.** The evaluation of novel therapeutic agents as well as novel delivery routes that bypass the blood-brain-barrier (e.g. convection enhanced delivery (CED)) in such preclinical models may be predictive of responses in human clinical trials, and may result in progress against BSGs.

Specific Aims

1. To determine the *in vitro* activity of a PDGFR-α neutralizing antibody in cell-lines derived from PDGF-B driven BSG

2. To evaluate the antitumor activity of systemic therapy with a PDGFR-α neutralizing antibody in the PDGF-B driven BSG mouse model

3. To evaluate the antitumor activity of convection-enhanced delivery (CED) with a PDGFR-α neutralizing antibody in the PDGF-B driven BSG mouse model

Background-

BSGs account for 15-20% of pediatric brain tumors and are the leading cause of death for children with brain tumors. The median survival for these children is less than 1 year after diagnosis. Despite decades of clinical trials evaluating novel agents to treat this disease, the natural history has not been significantly affected and 90% of children die within 2 years of diagnosis. Involved-field fractionated radiation to a total dose of 54Gy is the current standard of care for these tumors – however, this treatment modality unfortunately provides only temporary relief of symptoms and has major side effects. Recent genomic analysis of human BSGs have unraveled that the most commonly reported genetic alteration is platelet-derived growth factor receptor alpha or PDGFRα, which is amplified in 30-40% of BSGs and overexpressed in 67% (Becher et al. 2010, Zarghooni et al. 2010)

Clinical Significance-

If systemic treatment or direct treatment using convection-enhanced delivery of a monoclonal neutralizing antibody targeting murine PDGFRα demonstrates a statistically significant survival benefit in the platelet-derived growth factor-B (PDGF-B) driven BSG mouse model, we are committed to working towards translating results from this proposal into a phase I study for children with BSG. It is worth noting that a similar neutralizing antibody from Imclone, which inhibits **human** PDGFRα (IC50 < 1nM), is already in clinical trials for adult gliomas as an intravenous infusion (Loizos et al. 2005), and this latter antibody can be readily translated into a phase I clinical trial to treat children with BSG.

Section 3.

i. Hypothesis

A genetically engineered brainstem glioma murine model that is driven by PDGF signaling (as a model for PDGFRα amplified BSG) may serve as a useful preclinical tool for the evaluation of an antibody inhibiting the activation of PDGFRα. The evaluation of novel therapeutic agents as well as novel delivery routes that bypass the blood-brain-barrier (e.g. convection enhanced delivery (CED)) in preclinical models may be predictive of responses in human clinical trials, and may result in progress against BSGs.

ii. Specific Aims

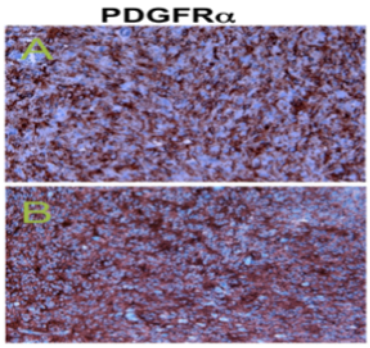
1. To determine the *in vitro* activity of a PDGFR-α neutralizing antibody in cell-lines derived from PDGF-B driven BSG

2. To evaluate the antitumor activity of systemic therapy with a PDGFR-α neutralizing antibody in the PDGF-B driven BSG mouse model

3. To evaluate the antitumor activity of convection-enhanced delivery (CED) with a PDGFR-α neutralizing antibody in the PDGF-B driven BSG mouse model

iii. Background and Rationale

Figure 1

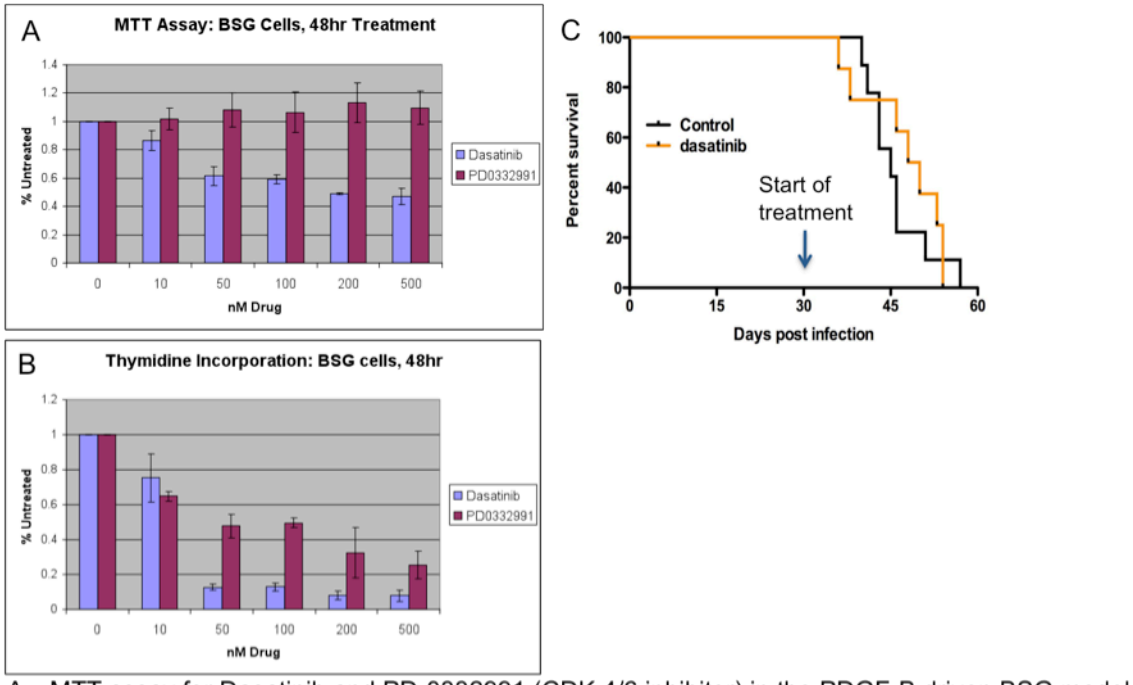


A-Human brainstem glioma **B**-Mouse brainstem glioma

Brainstem gliomas or diffuse intrinsic pontine gliomas are incurable tumors that arise in children. Thirty years of clinical trials have failed to identify a single agent that can increase the survival of children with brainstem gliomas. Dr. Becher and colleagues has developed the first genetically engineered mouse model of BSG based on molecular alterations documented in human tumors. This model harbors germ-line loss of the tumor suppressor Ink4a/ARF, and directed over-expression of the oncogene **PDGF-B** to Nestin-expressing cells of the brainstem (Becher et. al 2010). Tumors from the mouse model overexpress PDGFRα in every tumor cell (Figure 1). This model recapitulates the genetic alterations of a subset of the human disease as PDGFRα is amplified in at least 30% of pediatric brainstem gliomas and the receptor is over-expressed in approximately 67% of human tumors as measured by immunohistochemistry (n=22; Figure 1). Platelet-derived growth factor-B (PDGF-B) is an important mitogen for glial progenitors and is a ligand for both platelet-derived growth factor receptor alpha and beta (PDGFRα and PDGFRβ). We have observed that inhibition of PDGFRα may be a therapeutic strategy in the BSG mouse model as *in vitro* inhibition of PDGFRα with Dasatinib (an inhibitor of PDGFRα, Src and Abl) in a cell-line derived from the BSG mouse model has resulted in growth inhibition with an IC50 around 200nM (Figure 2A, 2B). Surprisingly, Dasatinib treatment of the PDGF-driven BSG mouse model *in vivo* (10mg/kg IP for one week; Figure 2C) has not resulted in a survival benefit even though Dasatinib treatment at similar doses have shown efficacy in mouse models of CNS leukemia (Lagas JS 2009; Porkka K 2008). The lack of efficacy of Dasatinib *in vivo* despite promising *in vitro* growth inhibition suggests that perhaps drug delivery is an issue or alternatively that *in vivo*, PDGFR-α signaling is not necessary for BSG tumor maintenance. One way to answer this question is to evaluate inhibition of PDGFRα in a delivery method that bypasses the blood-brain-barrier (BBB). Convection-enhanced delivery is a neurosurgical technique where the therapeutic agent is infused directly into the tumor via a catheter and it is thought to bypass the blood-brain-barrier. In this proposal, we are going to evaluate this neurosurgical approach with an antibody that blocks

PDGFR-α signaling (Russell MR et al. 2010). As we already have evidence that inhibition of PDGFR-α has antitumor activity *in vitro*, this proposal will help us better understand whether the relatively intact BBB is be the reason for the lack of efficacy in vivo.

Figure 2



A. MTT assay for Dasatinib and PD-0332991 (CDK 4/6 inhibitor) in the PDGF-B driven BSG model
B. Thymidine incorporation assay for Dasatinib and PD-0332991
C. Survival analysis of BSG-bearing mice treated with one week of 10mg/kg IP Dasatinib

Convection-enhanced delivery (CED)

The local delivery of therapeutic agents has been explored for the treatment of malignancies, cellular enzyme deficiencies, and other pathological conditions, which present a need for highly specific treatment modalities. Convection-enhanced delivery (CED) lowers the likelihood of systemic side effects and toxicities, increases local effective concentrations, and provides a controlled infusion of therapeutic agent to a tissue target (Bobo et al. 1994; Lidar et al. 2004; Morrison et al. 1994). CED is based on the principle of bulk flow to carry an infusate into parenchyma directly across the blood-brain barrier (BBB), which has been a physical barrier to the systemic delivery of agents. The concentration of the therapeutic solute has the potential for reaching values 10,000x greater than intravenous administration (Groothuis et al. 1999). The additional benefit of CED is the intercalation of the infusate between white matter tracts in the space where the glioma cells exist (Raghavan et al. 2006; Figure 3 below is an illustration from this reference).

Stereotactic placement of catheters into brain parenchyma has been studied in several animal models, including primates and rats. The emergent neurologic functions of animals treated briefly as well as over a prolonged period of time have remained stable based on simple scoring systems (Sandberg et al. 2002).

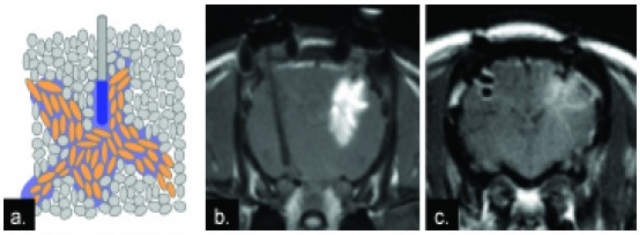


FIG. 3. a: Sketch illustrating an infusion catheter in tissue (not to scale). Orange elongated cells represent white matter tracts. The fluid infused from the catheter forms a small annulus around the outside of the catheter, the backflow. This cylinder is the source of the subsequent infusion, which preferentially follows the white matter tracts. b: A T_1-weighted MR image demonstrating the infusion of Gd-DTPA into a pig brain. The infusion pattern has an irregular shape, preferentially following the white matter tracts. The image was acquired at the end of the infusion. c: A T_1-weighted MR image obtained 1 day after the infusion was finished, depicting the effects from the same infusion shown in panel b. The Gd-DTPA has diffused to distances far beyond the original volume shown in panel b.