STANFORD UNIVERSITY MEDICAL CENTER
DEPARTMENT OF NEUROLOGY AND NEUROLOGICAL SCIENCES
DIVISION OF CHILD NEUROLOGY

STANFORD UNIVERSITY
SCHOOL OF MEDICINE

LUCILE SALTER PACKARD
CHILDREN'S HOSPITAL

7/20/2012

Dear Mr. Desserich and The Cure Starts Now Foundation,

Enclosed, please find our research proposal entitled A Combinatorial Approach to Target Cellular Subpopulations in Diffuse Intrinsic Pontine Glioma for your consideration. Included in a single PDF document are the proposal, budget, my CV and a collaborator's letter of support from Dr. Charles Keller. Please let me know if there is any additional information you would like me to provide.

Thank you again.

Sincerely,

Michelle Monje, MD PhD
Assistant Professor of Neurology and Neuro-Oncology

750 Welch Road, Suite 317 • Palo Alto, CA 94304
TELEPHONE: 650.736.0885 • FAX: 650.723.7299

A Combinatorial Approach to Target Cellular Subpopulations in Diffuse Intrinsic Pontine Glioma

Specific Aims

Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating childhood cancer. We have recently developed the first available cell culture and orthotopic xenograft models of DIPG [1]. These invaluable resources, together with our parallel studies of normal brainstem development, have led to the discovery of a DIPG tumor-initiating cell type. The tumor-initiating cell, or "cancer stem cell (CSC)", of DIPG is a small subpopulation that is responsive to the powerful signaling pathway Hedgehog[1]. The classic cancer stem cell hypothesis posits that the cancer stem cell is solely responsible for tumor propagation, as though the CSC population is like the tumor's "engine", giving rise to a rapidly proliferating transit amplifying cell type and more "differentiated" cell types downstream in the cellular hierarchy. Without the CSC, all daughter cell types would be expected to burn out and the tumor to become indolent.

We have targeted the DIPG tumor initiating cell population using pharmacological Hedgehog pathway inhibition. We have now shown that while Hedgehog pathway inhibition has dramatic effects on tumor cell self-renewal *in vitro*[1], and does slow tumor growth for about a month *in vivo*, it does not change the ultimate size of the tumors that diffusely infiltrate the brainstem in our DIPG orthotopic xenograft model nor does it significantly alter survival. Hedgehog inhibitor therapy does, however, appear to deplete or eliminate the tumor-initiating cell population *in vivo*. In other words, the "cancer stem cell" is gone, but the tumor still grows enough to kill the mouse. It may be that DIPG tumor cell behavior does not conform precisely to the cancer stem cell hypothesis, or it may be that in DIPG the time required for transit amplifying cell "burn out" is not tolerated by the functionally critical, relatively small and confined brainstem. Either way, these findings point to a paradigm shift from the classic cancer stem cell hypothesis, and so a novel strategy is needed. **We thus propose to test the hypothesis that it is necessary to target multiple cellular subpopulations to achieve a survival benefit in DIPG.**

AIM I Define the cellular heterogeneity of primary DIPG tumor samples using single cell molecular analyses To clearly define distinct cellular compartments within DIPG, and simultaneously discover new molecular targets, we will perform a single cell gene expression analysis and subsequent classification of cellular subpopulations based on gene expression signatures. New microfluidic technology allows effective, high throughput single cell analysis of quantitative gene expression. Once defined, the distinct subpopulations of cells will be isolated using a FACS strategy based on cell surface marker expression.

AIM II High throughput *in vitro* drug screening of each cellular subpopulation This Aim builds upon the Cure Starts Now DIPG Preclinical Consortium project. To target the molecularly distinct cellular subpopulations within the heterogeneous tumor, we will screen each subpopulation individually. Each subpopulation will be isolated from primary xenografts using a FACS strategy developed in AIM I, and screened using drug plates developed by Dr Charles Keller. The advantage of this strategy is that it may capture important drug targets not appreciated by the more standard method of screening neurosphere cultures, as neurosphere cultures are enriched for one particular cellular subpopulation and fail to model the true diversity of DIPG tumor cell types in the endogenous tumor.

AIM III *in vivo* preclinical testing and development of a combinatorial strategy to target the functional cellular compartments in DIPG We can target the DIPG tumor-initiating cell ("CSC") using Hedgehog pathway inhibitors, and while this is presumably necessary to prevent recurrence or spread, it is not sufficient to prolong survival. The experiments described below are designed to identify and test the best combination of molecular targets in order to subdue or eliminate the DIPG cellular subpopulations responsible for DIPG lethality.