



University of California San Francisco Brain Tumor SPORE Update

Spring 2004

...A Message From The Director

The Specialized Program of Research Excellence (SPORE) grant has created unprecedented opportunities for applied researchers and basic science investigators in the Brain Tumor Research Center and Comprehensive Cancer Center at the University of California San Francisco (UCSF) to work together in a supportive environment within the UCSF campus that did not exist in such a robust fashion prior to this award.

A fine example of this collaborative spirit was the first UCSF Joint Brain, Breast, and Prostate SPORE Scientific Retreat, held in February 2004. Brain SPORE investigators gave four presentations: three from ongoing SPORE projects and one from emerging research. **Andrew Parsa MD, PhD** presented his progress in the development of a clinical tumor-vaccine protocol based on preclinical investigation using the FL +3 ligand/heat shock protein construct. A clinical trial protocol from this project will be submitted to the protocol review committee of the UCSF Comprehensive Cancer Center by the end of 2004. **Krys Bankiewicz MD, PhD** presented his work with convection-enhanced delivery (CED) in animal systems, which has used magnetic resonance imaging and labeled liposomes with encapsulated gadolinium to determine drug distribution in different brain regions after CED. Based on work with **John Park MD**, a gadolinium/doxorubicin (DOXIL) liposome was infused into rat brains using CED, and demonstrated complete coverage in the 9L-2 invasive glioma model. The third presentation was given by **Joseph Costello PhD**, on the topic of hypomethylation-induced reactivation of oncogenes in glioblastoma, which could potentially provide a new group of growth-promoting gene targets for therapy for high-grade glioma. The fourth presentation, by **Margaret Wrensch PhD**, reviewed the results from the San Francisco Bay Area Adult Glioma Survival Study.

We are especially excited about the collaborative opportunities afforded by the new Cancer Research facility being constructed at UCSF. One entire floor (25,000 square feet) will be allocated to the Brain Tumor program, including all SPORE investigators. We will first move into 10,000 square feet of new space at Mission Bay next year and into our new center by the end of 2006 or early 2007. We expect that working in proximity to our fellow cancer researchers will lead to even more collaborative translational research opportunities in the future.

Mitchel S. Berger MD
Kathleen M. Plant Distinguished Professor
in Neurological Surgery
Director, UCSF Brain Tumor SPORE
and UCSF Cancer Center Neurologic Oncology Program



Funded Translational Projects and Investigators

San Francisco Bay Area Adult Glioma Survival Study

Principal Investigator: **Margaret Wrensch PhD**

Clinical Co-Principal Investigator: **Michael Prados MD**

Prognostic Value of MRSI Parameters for Patients with Glioma

Principal Investigator: **Sarah Nelson PhD**

Clinical Co-Principal Investigator: **Susan Chang MD**

Development of Novel Targeted Therapeutics for Brain Tumor Treatment

Principal Investigator: **John Park MD**

Clinical Co-Principal Investigator: **Mitchel Berger MD**

Exploiting the PI3-Kinase Pathway in Human Glioma Therapy

Principal Investigator: **David Stokoe PhD**

Clinical Co-Principal Investigator: **Daphne Haas-Kogan MD**

Career Development Awardees

Gabriele Bergers PhD

Hypoxia and Neovascularization: Cause or Consequence of Glioblastoma Multiforme Progression? Studies in Genetically Engineered Murine Tumor Models

Tracy Richmond McKnight PhD

Correlation of MRS Features of Glioma with Tumor Markers

Developmental Research Awardees

Andrew T. Parsa MD, PhD

Antigen-Specific Modeling of Glioma Immunotherapy

William A. Weiss MD, PhD

Combinatorial Therapy for Glioma

*New Career Development and Developmental Research Awardees Chosen for 2004-2005
see p. 4*

SPORE Career Development Research in Progress



William Weiss MD, PhD

Principal Investigator

Assistant Professor of Neurological Surgery, Neurology, and Pediatrics

Principal Investigator, BTRC

Project Title: Combinatorial Therapy for Glioma

Funding Agency: National Cancer Institute/SPORE

Co-Investigators and Collaborators: Kevan Shokat, Qi-Wen Fan, David Goldenberg, Zachary Knight, and Chao Zhang

Project Summary: The epidermal growth factor receptor (EGFR), a prototype receptor tyrosine kinase, is commonly amplified and over-expressed in gliomas. Relatively selective inhibitors of EGFR are being studied in clinical trials; however no template is available to guide the optimal use of such therapies, and more work needs to be done to identify agents that show combinatorial efficacy in the absence of combinatorial side effects. In addition, whether the apparent efficacy of a specific kinase inhibitor is attributable solely to inhibition of its primary target, or to combined inhibition of additional unidentified kinases, is a critical issue in the use of these targeted agents.

In preliminary experiments, we have introduced activated alleles of EGFR into tumor cell lines. Surprisingly, inhibition of EGFR in established cell lines led to proliferation arrest, associated with decreased levels of D- and A-type cyclins, and with decreased signaling through the PI3 kinase pathway. These data suggest that mammalian cells become dependent on aberrant oncogenic signaling; this dependency renders them incapable of returning to a normal proliferative phenotype.

Based on these studies, we hypothesized that dual inhibition of EGFR and PI3 kinase could show improved potency. We therefore established tumor xenografts from the human glioma cell line U87:MG transduced with Δ EGFR, an activated allele of *EGFR* that is commonly found in patients with glioma. We treated tumor-bearing mice with vehicle, the EGFR inhibitor ZD1839, the PI3 kinase inhibitor LY294002, or ZD1839 plus LY294002. In these experiments, inhibition of EGFR cooperated with inhibition of PI3 kinase to arrest growth of established tumors.

Our experiments provide a preclinical mechanistic basis for combining biologically based therapies directed against two targets within a complex signaling cascade. This cooperativity was observed using dosages for each compound that are significantly lower than those required for use as monotherapy, suggesting that similar combinatorial therapy applied to glioma patients may allow the use of relatively toxic agents at concentrations below those reported to elicit dose-limiting side effects. Our current experiments are analyzing the impact of inhibitors of EGFR in combination with inhibitors of signaling molecules that signal downstream of PI3 kinase. We are using a combination of small molecule inhibitors and regulated delivery of siRNA to accomplish these goals.

Recent Publication

Fan QW, Specht KM, Zhang C, Goldenberg DD, Shokat KM, Weiss WA. Combinatorial efficacy achieved through two-point blockade within a signaling pathway—a chemical genetic approach. *Cancer Res* 2003;63:8930-8.

Featured Translational Research Project



Development of Novel Targeted Therapeutics for Brain Tumor Treatment

Principal Investigator: John Park MD

Clinical Co-Principal Investigator: Mitchel Berger MD

Malignant brain tumors pose unique and challenging problems for treatment. High-grade astrocytic tumors such as glioblastoma multiforme (GBM) are usually incurable. Surgical approaches are not curative largely because of infiltrative growth into surrounding normal brain tissue. Radiation therapy can be used to treat the involved region, but is limited by inadequate tumor specificity and by the inherent radiation resistance of these tumors. Similarly, conventional chemotherapy is severely limited by lack of specificity and the inability to achieve effective drug concentrations within the brain without causing excessive systemic toxicity. Newer, more targeted therapies are needed for brain-tumor treatment. Better therapies must achieve efficient delivery of agents not only to the brain but—by selective and efficient targeting—to the tumor cells themselves. The goal of such therapy is to target tumor cells throughout a region of brain tissue without damaging normal cells.

Unlike most immunotoxins used in cancer therapy, drug-loaded immunoliposomes provide an additional level of specificity through the delivery of cytotoxic drugs with their own selectivity for cancer cells, and, in the case of approved chemotherapeutics, an established (although narrow) therapeutic index. Antigen expression in target cells needs only to be relatively higher than in normal cells. Immunoliposomes provide both direct targeting of tumor cells and bystander killing by the diffusion of small-molecule drugs to adjoining tumor cells. Importantly, immunoliposomes can be constructed to be long-circulating and non-immunogenic. In this project, liposomes will be designed to carry various toxic small molecules or nucleic acid constructs that are potent agents against brain tumors. These liposomes will then be targeted to glioma cells by linkage to antibody fragments specific for tumor cells expressing epidermal growth factor receptor (EGFR) or mutant EGFR.

Because of the problems associated with systemic administration of therapeutic agents, such as the blood-brain barrier, strategies for local administration into the brain have been the default mode for many agents. Convection-enhanced delivery (CED) uses a pressure gradient established at the site of an infusion catheter to “push” both drug and solvent through the extracellular space. CED can achieve relatively homogeneous distribution of drugs over considerable distances in the brain. In close collaboration with Krys Bankiewicz MD, PhD (University of California San Francisco (UCSF) Brain Tumor Research Center), these immunoliposomes will be administered into the brain by using CED.

We have several specific aims for this project:

1. Evaluate anti-EGFR immunoliposomes for targeted delivery of anticancer agents, such as:
 - a. Small molecule drugs: doxorubicin, novel compounds (e.g., ellipticine, breflato)
 - b. Nucleic acid-based constructs in genospheres
2. Construct immunoliposomes targeted to EGFR-associated brain tumors.
3. Optimize immunoliposomes in conjunction with regional delivery methods in preclinical models of glioma.
4. Perform advanced preclinical studies and clinical development of the best construct.

In the past year, we have made great progress in achieving the specific aims of our project. We have optimized the stability and expression of EGFR-targeted immunoliposomes, and have used CED to achieve extensive and efficient distribution of liposomes within normal mouse brains. To our knowledge, this is the first report of CED infusion of liposomes into the central nervous system. These liposomes were also used to deliver imaging agents for magnetic resonance imaging-guided CED. In addition, we have developed a new and highly robust method for encapsulation of a number of novel drug classes into liposomal/nanoparticle agents; each of these novel liposomal drugs can be converted to an immunoliposome version using established technologies. Based on our preclinical studies, we propose to develop liposomal vinorelbine for translation to clinical development.

<http://neurosurgery.medschool.ucsf.edu/>

New Career Development Awardees



Tumor-associated inflammation as a new target for glioma therapy
Nalin Gupta MD, PhD
Department of Neurological Surgery,
University of California,
San Francisco



The development of novel therapeutic agents directed towards preventing tumor recurrence in malignant gliomas
Fredrick Gorin PhD
Department of Neurological Surgery,
University of California, Davis

New Developmental Research Awardees



New targets for therapy of glioblastoma multiforme unmasked by demethylation
Joe Costello PhD
Department of Neurological Surgery,
University of California,
San Francisco



Identification of chimeric transcripts in brain tumors using end sequence profiling
Collin Collins PhD
Cancer Research Institute,
University of California,
San Francisco



Determining and predicting tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) sensitivity in primary glioblastoma multiforme
Russ Pieper PhD
Department of Neurological Surgery,
University of California,
San Francisco