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validated. Studies aimed at identifying chimeric transcripts revealed approximately 20 cDNA clones that were predicted to encode chimeric genes; PCR validation of these clones confirmed that three were expressed in the tumor. The experimental validation of these three chimeric transcripts, coupled with computational evidence for many more, suggests that the genomes of brain tumors may encode significant numbers of chimeric transcripts. If confirmed in tissue from additional tumors, these results could have important implications for targeted therapeutics and immunotherapeutic approaches.

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exhibiting subG1 DNA content (a measure of apoptosis) following 24-hour exposure to 0–1000 ng/ml TRAIL. The cells are dissociated, expanded in short-term culture, verified as GBM by comparative genomic hybridization, and assayed by fluorescence-activated cell sorting. These GBM samples are then assayed for expression of FLIP, phosphorylated (activated) Akt, and phosphorylated mTOR by using Western blotting. The concentration of FLIP is compared to TRAIL sensitivity to confirm an inverse relationship.

To determine if inhibition of the Akt-mTOR pathway sensitizes primary GBM to TRAIL-induced apoptosis, aliquots of the short-term cultured GBM cells are incubated with the mTOR inhibitor rapamycin. The cells are then exposed to TRAIL and monitored for levels of phospho-mTOR and TRAIL sensitivity.

The data derived from these studies may prove useful in stratifying patients with GBM for clinical trials of convection-enhanced delivery of TRAIL, as well as helpful for defining methods of circumventing TRAIL resistance using clinically approved mTOR inhibitors.



## University of California, San Francisco Brain Tumor SPORE Update Spring 2005

### ...A Message From the Director

The University of California, San Francisco's (UCSF) Neurologic Oncology Program was awarded a Specialized Program of Research Excellence (SPORE) grant in part due to its history of original and significant research into new treatments for brain tumors. The Comprehensive Cancer Center and the Brain Tumor Research Center at UCSF have a long tradition of true bench-to bedside translational research—research in which scientists and clinicians work in partnership to rapidly translate laboratory findings into new or improved forms of therapy. The format of the SPORE grant was designed to accelerate this process. Projects proposed for a SPORE must be collaboratively designed and executed by physicians experienced in patient-oriented research in tandem with either basic scientists working at the cellular and molecular levels or population scientists experienced in studying the patterns of disease.



One area of research that has proven particularly amenable to rapid translation to the clinical setting has been the development of small-molecule inhibitors. These drugs target aberrant cell-signaling pathways in brain tumors, blocking cell division instead of causing apoptosis. Although traditional cytotoxic drugs can be effective in slowing tumor progression, they can sometimes cause severe side effects because they do not target tumor cells specifically; any dividing cell in the body can be destroyed. Small-molecule inhibitors have a greater specificity for tumor cells, and therefore produce fewer side effects. Investigators David Stokoe PhD and Daphne Haas-Kogan MD, who are leading one of our SPORE projects, have been examining potential small-molecule inhibitors of the phosphoinositide-3-kinase (PI3-kinase) signaling pathway, which is often dysregulated in malignant gliomas. The investigators analyzed the results from a phase I clinical trial of erlotinib (Tarceva) in patients with malignant glioma, and found that patients whose tumors expressed high levels of epidermal growth factor receptor (EGFR) and low levels of phosphorylated PKB/Akt, both components of the PI3-kinase pathway, had the best response to erlotinib. This study was recently published in the *Journal of the National Cancer Institute*, and will be used to design a phase II trial testing the efficacy of erlotinib in patients with malignant glioma. Selecting treatment for individual patients based on the molecular characteristics of their tumors is an outstanding example of how the SPORE grant is translating molecular research into improved care for patients.

Mitchel S. Berger MD  
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

#### Nalin Gupta MD, PhD

Tumor-associated inflammation as a new target for glioma therapy

#### Fredric Gorin PhD

The development of novel therapeutic agents directed towards preventing tumor recurrence in malignant gliomas

### Developmental Research Awardees

#### Joseph Costello PhD

New targets for therapy of glioblastoma multiforme unmasked by demethylation

#### Colin Collins PhD

Identification of chimeric transcripts in brain tumors using end sequence profiling

#### Russell Pieper PhD

Determining and predicting tumor necrosis factor-related apoptosis-inducing ligand sensitivity in primary glioblastoma multiforme

## SPORE Developmental Research in Progress



**Colin Collins PhD**  
**Principal Investigator**

*Assistant Professor, Cancer Research Institute and Laboratory Medicine*  
*Program Member, University of California, San Francisco Comprehensive Cancer Center*

**Project Title:** Identification of Chimeric Transcripts in Brain Tumors Using End-Sequence Profiling

**Project Summary:** Genomic instability is a hallmark of cancer, and cytogenetic techniques have revealed that brain-tumor genomes carry large numbers of translocations and other complex rearrangements. In an effort to identify chimeric genes that are created from the fusion of two or more normal genes and produce a functional open reading frame, end-sequence profiling (ESP) can be used to perform structural analysis of the genomes and transcriptomes of brain tumors. While established cytogenetic methods such as array comparative genomic hybridization (aCGH) can reveal structural aberrations, their lack of resolution and limited integration with the genome sequence makes identification of specific chimeric genes extremely difficult. To overcome these problems, the Collins laboratory is refining ESP, a technology that allows high-output mapping and simultaneous cloning of all genome rearrangements. ESP has so far been limited to the identification of chromosomal structural rearrangements and has not been able to directly identify the resulting chimeric mRNA transcripts. In this project, ESP will be modified to allow for analysis of transcriptomes, so that chimeric transcripts can be mapped to chimeric genomic clones *en masse*. Successful modification of ESP will allow for the positive identification of presumed drug targets and associated predictive biomarkers.

Research so far has been performed on tissue from a recurrent tumor (glioblastoma multiforme) for which aCGH and genome-wide methylation data were available. Chromosome copy-number profiles obtained by using aCGH and ESP showed excellent correlation to each other. Numerous likely interchromosomal rearrangements were identified and are now being

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**Russell Pieper PhD**  
**Principal Investigator**

*Associate Professor of Neurological Surgery*  
*Principal Investigator, Brain Tumor Research Center*

**Project Title:** Determining and predicting tumor necrosis factor-related apoptosis-inducing ligand sensitivity in primary glioblastoma multiforme

**Project Summary:** This project aims to improve therapy for glioblastoma multiforme (GBM) by understanding the resistance of these tumors to the molecule tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). The 60 kD TRAIL protein is an attractive therapeutic molecule because it induces apoptosis in tumor cells, but not in normal cells. While many tumor types are TRAIL sensitive, GBM are largely TRAIL resistant. This resistance is not a result of down regulation of TRAIL receptors, but rather appears to be the result of alterations in the pathway that connects activated TRAIL receptors to the apoptotic machinery.

Aberrant apoptosis could be the result of elevated levels of fas-associated death-domain-like IL-1 $\beta$ -converting enzyme inhibitory protein (FLIP), which blocks apoptosis by binding to caspase-8 following TRAIL-receptor activation. Preliminary data has shown that TRAIL sensitivity of GBM cell lines inversely correlates with expression of FLIP. FLIP expression is, in turn, increased by Akt via activation of the protein mammalian target of rapamycin (mTOR). This suggests a means by which Akt-overexpressing GBM may resist TRAIL-induced apoptosis. All previous studies of these mechanisms have been performed in cultured GBM cell lines, and this is the first study to examine TRAIL sensitivity in primary brain tumors. Investigators are examining whether or not inhibition of the Akt-mTOR pathway will suppress levels of FLIP to the point that GBM will become sensitive to TRAIL.

In order to test TRAIL sensitivity in a panel of primary GBM samples, investigators are measuring the percentage of cells

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## Featured Translational Research Project



### San Francisco Bay Area Adult Glioma Survival Study

*Principal Investigator: Margaret Wensch PhD*

*Clinical Co-Principal Investigator: Michael Prados MD*

Developing more sophisticated prognostic indicators for glioma and refining tumor classification within and between histological subtypes is essential for developing targeted therapies, and for providing effective therapy to individual patients while sparing them potentially unnecessary treatment. This project is making progress toward these goals by analyzing survival in relationship to both established and novel prognostic factors.

Investigators began by determining the vital status for 873 patients studied in two population-based case series conducted in the San Francisco Bay Area between 1991-1994 (series 1) and between 1997-1999 (series 2) as part of a previous study by Margaret Wensch PhD. After collecting these data, investigators began analyzing the patients' survival, evaluating and controlling for variables such as age at diagnosis, ethnicity, tumor histopathological type and grade, and whether or not patients received chemotherapy or radiation therapy. The project is also examining patients' survival as a function of various potential tumor markers; p53 and p53 mutation analyses, and p53 IHC, MDM2, and EGFR amplification studies on biopsy samples from 500 patients with astrocytomas have been completed.

Other aims of the project have expanded to include promising collaborations with other researchers at UCSF, as well as fellow SPORE recipient University of Alabama, Birmingham (UAB) and the nonprofit organizations Accelerate Brain Cancer Cure (ABC2) and the National Brain Tumor Foundation (NBTF). Investigators from Project 1 have combined their efforts with Project 4 principal investigators Daphne Haas-Kogan MD and David Stokoe PhD to study PTEN methylation in relation to high-grade and low-grade gliomas. They have found that PTEN is methylated in 55% of low-grade tumors, but in only 9% of glioblastoma multiforme (GBM), suggesting that a pathway for PTEN dysregulation other than loss and mutation exists. Based on these findings, PTEN methylation is being examined as a favorable prognostic indicator in GBM.

A second focus of tumor-marker investigation has fostered collaboration with Andrew Parsa MD, PhD and Tarik Tihan MD, PhD at UCSF, who are working to characterize a variety of gliomas with respect to CD8 tumor infiltrates. Wensch chose 40 patients from the San Francisco Bay Area Adult Glioma Study (SFBAAGS) for CD8 analysis. Initial progress has shown that 35.5% of long-term survivors have increased amounts of CD8 tumor infiltrates, compared with only 12.5% of short-term survivors. These analyses suggest that CD8 tumor infiltrates might be a worthwhile prognostic indicator, and additional patients are being selected for further study in the coming year.

In addition to examining the biology of tumor markers, the project has also made progress in genotyping. Researchers have collaborated with colleagues at UAB to genotype human lymphocyte antigens in blood specimens from patients from the SFBAAGS. Results have shown that GBM is positively associated with HLA genotypes/haplotypes B\*13 and B\*07-Cw\*07 and inversely associated with Cw\*01, while HLA A\*32 and B\*55 were associated with length of survival.

In conjunction with SPORE funding, Wensch, Prados, and colleagues have also received funding from ABC2 and the NBTF to perform a large-scale genotyping of single-nucleotide polymorphisms in relation to survival and glioma case-control status. They have genotyped 112 patients with glioma and 112 individuals in a control group and have found several promising candidate genes for further exploration.

In the last year, a substantial amount of data has been obtained about the most promising variables that could serve as prognostic indicators for glioma. Investigators have made excellent progress in collecting and categorizing these data, and are now in the process of analyzing them for population science, basic science, and clinical value.

The SPORE grant awarded to UCSF funds four major translational research projects, each driven by a pair of applied and basic researchers, and each intended to create novel tools and therapies potentially useful in the treatment of human brain tumors. In addition, a Career Development Research Program included in the SPORE supports new investigators in the field, and a Developmental Research Program provides initial funding of promising projects that may develop into future SPORE projects.