1. Scientific Abstract

About 18,400 people per year in the United States are diagnosed with a malignant primary brain tumor, the majority of which are gliomas, and over 12,000 people a year die of the disease. Treatment failure of gliomas is largely attributable to the inability of current strategies to address the diffuse and highly infiltrative nature of these tumors. Due to extensive dissemination of these aggressive tumor cells away from the primary site into the surrounding brain, there is no clear border between tumor edge and normal brain, with invasive cells and distant micro-foci proving exceptionally difficult to treat by surgical resection, irradiation, chemotherapy or gene therapy. Drug delivery has been ineffective due to an inability for many drugs to cross the blood-brain barrier and/or poor diffusion of drugs within the brain and within the tumor. An inability to deliver therapeutic agents with sufficient distribution throughout primary and distant tumor sites limits the effectiveness of gene therapy. Immunotherapeutic strategies have also failed to show significant impact at the clinical level, largely due to their inability to eradicate disseminated tumor foci throughout the brain (Ehtesham *et al.*, 2005). Despite aggressive treatments, the recurrence rate for glioma is 98% – with 80% occurring within 2 centimeters of the resection cavity. Median survival for these patients is only 3-6 months. To improve clinical outcome, novel therapies must address the invasive tumor cells that escape currently available treatments, while limiting toxicity to normal tissues.

Neural stem/progenitor cells (NSCs) display inherent tumor-tropic properties that can be exploited for targeted delivery of anti-cancer agents to invasive and metastatic tumors. We postulate that this NSC-mediated treatment approach will provide increased tumor-selective, localized treatment with decreased exposure of normal tissues to the associated toxicities. This would potentially achieve therapeutic indices sufficient to eradicate invasive tumors that are otherwise lethal. Significant anti-tumor responses, as measured by decreased tumor burden, or increased survival time have been achieved with various therapeutic transgenes, in numerous orthotopic brain tumor models including glioma, solid tumor metastases to brain, medulloblastoma, and disseminated neuroblastoma.

We propose the clinical use of a well-characterized, clonal human NSC line, HB1.F3, that has been modified to express a therapeutic transgene for the treatment of patients with recurrent high-grade glioma. The HB 1.F3 human NSC line has been extensively studied for determination of its therapeutic potential in animal tumor models of glioblastoma, medulloblastoma, and disseminated neuroblastoma. In rodent models of orthotopic human brain tumors, we have demonstrated that HB1.F3 NSCs can selectively target invasive tumor cells and distant tumor micro-foci. Even when injected into the hemisphere contralateral to the tumor, they migrate through the brain parenchyma and across white matter tracts to localize to the tumor site. In all invasive and metastatic solid tumor models, 70-90% therapeutic efficacy was achieved as measured by increased long term survival or decreased tumor burden. Preliminary biodistribution studies of the HB1.F3 NSCs indicate this cell line is safe, non-immunogenic and non-tumorigenic. Furthermore, these cells retain their tumor-tropic property when modified to express therapeutic transgenes. We believe that using a stable, sustainable, and expandable NSC line will circumvent the problems associated with characterization, senescence, and replenishment sources of primary stem cell pools. In the current proposal, we postulate that NSCs can deliver the enzyme, cytosine deaminase (CD), to tumor foci and convert systemically administered prodrug 5-fluorocytosine (5-FC) to the chemotherapeutic 5-fluorouracil (5-FU). Thus, 5-FU, which is selectively toxic to dividing cells, will be produced and concentrated at tumor foci. Of note, 5-FU does not cross the blood-brain barrier, however 5-FC is known to cross very well (levels of 5-FC in the CSF of human patients were approx. 85% that measured in blood plasma) (Brouwer *et al.*, 2007). Since human enzymes do not convert 5-FC to 5·fU, we envision that 5-FC, when given systemically, will distribute into the CSF and be converted to 5-FU selectively at tumor sites to which CD-expressing NSCs have migrated. We expect this strategy will limit toxicity to non-tumor tissues and improve clinical outcome for glioma patients. A pilot feasibility study to assess the safety of intracerebrally administered CD-expressing HB 1.F3 NSCs in combination with oral 5-FC in patients with recurrent high-grade gliomas is currently under development.

REFERENCES

1. Ehtesham. M., Stevenson. C. B., and Thompson, R. C. Stem cell therapies for malignant glioma. Neurosurg Focus, *19*: E5, 2005.
2. Brouwer. A. E., van Kan, H. J., Johnson, E., Rajanuwong, A., Teparrukkul, P., Wuthiekanun, Y., Chierakul, W., Day, N., and Harrison, T. S. Oral versus intravenous flucytosine in patients with human immunodeficiency virus-associated cryptococcal meningitis. Antimicrob Agents Chemother, *51*: 1038-1042. 2007.