

# Cell-free DNA in patients with Glioblastoma Multiforme: A Pilot Study

Leif-Erik Bohman

Primary Investigator: Jeffrey Bruce, MD

## 1. Study Purpose & Rationale

### *Background*

Glioblastoma Multiforme (GBM) the most common primary brain tumor in adults and carries a dismal prognosis despite aggressive surgical or medical intervention. It accounts for roughly fifteen percent of all brain tumors and fifty to sixty percent of primary brain tumors (ABM Salah Uddin 2005). Though there are radiographic characteristics which are suggestive of GBM, a definitive diagnosis requires tissue biopsy. Surgical biopsy can be achieved with either a craniotomy or stereotactic surgery. Craniotomy allows excision of the tumor, however surgery is not curative and the benefits of cytoreduction must be carefully weighed against the risks. Craniotomy for brain tumor has a risk of post operative hemorrhage of roughly one percent, a two percent risk of wound infection, and a ten percent risk of worsening of neurologic deficits in the first twenty-four hours after surgery. Stereotactic biopsy carries reduced risk, however there is still danger of post-operative infection or worsening of neurological deficits, particularly if the tumor lies in an eloquent area (Greenberg 2006). For these reasons, a non-invasive diagnostic test for GBM would be valuable, offering patients a means to avoid the risks of biopsy.

One of the most commonly examined and easily assessed types of genetic alterations present in cancer is loss of heterozygosity (LOH) on particular chromosomes and this can be assessed from cell free serum with a combination of DNA amplification and PCR labeling (Taback and Hoon 2004). In a large, population-based study of GBM, Ohgaki *et al* identified a number of common genetic markers present in patients histologically diagnosed with GBM, with some markers present in as many as 71% of these patients. These markers included LOH at 10q, *EGFR* amplification, *TP53* mutations, p16<sup>INK4α</sup> deletion, and *PTEN* mutations (Ohgaki, Dessen *et al.* 2004). Similarly Hill *et al* studied 98 consecutive cases of GBM resected over a 4 year period and found similarly that LOH at 10q, *EGFR* amplification, *PTEN* mutation, and *TP53* mutations were common with frequencies of individual alterations as high as 76% as well as noting common mutations in LOH 10p, LOH 1p, and LOH19q with frequencies up to 67% (Hill, Hunter *et al.* 2003).

Cell free DNA has been shown to be present in patients with various types of neoplasms and has been quantitatively shown to be significantly elevated in the blood of patients with solid tumors compared to patients with nonneoplastic diseases (Shapiro, Chakrabarty *et al.* 1983). This cell free DNA is not thought to be directly related to metastasis and instead is thought to be released early in tumor growth during apoptosis and necrosis of tumor cell. Moreover, this cell free DNA may be analyzed for specific genetic markers of neoplasm with varying degrees of specificity and sensitivity. In bladder cancer, one study showed that specific DNA alterations could be detected by PCR with a combined sensitivity and specificity of roughly 80% compared to controls (Von Knobloch *et al* 2004). Other investigators have found loss of heterozygosity (LOH) in 38-46% of a sample of patients with primary and metastatic breast cancer, even in the absence of circulating breast cancer cells (Schwarzenbach, Muller *et al.* 2004). GBM is characterized by a high level of necrosis and the cells may release DNA into the surrounding interstitium.

The central challenge to this study is that even if DNA is being released by GBMs (which we believe to be probable given the high levels of necrosis and aggressive growth patterns which

are characteristic of these tumors) the blood-brain barrier may prevent this DNA from passing in significant quantity into the peripheral circulation, thus making such assays impossible. Indeed, one recent study failed to demonstrate the presence of tumor cells in the peripheral circulation by means of RT-PCR for GFAP mRNA (Bohm, Wassmann et al. 2003). Though it seems plausible that mRNA would be less stable than DNA in the plasma, telomerase mRNA has been shown to be detectable in the serum of patients with breast cancer (Chen, Bonnefoi et al. 2000). A study on healthy individuals showed remarkable stability of *GADPH* mRNA after incubation or freezing and thawing, however as these authors point out, different mRNA molecules may have different stabilities and the results may not be generalizable (Tsui, Ng et al. 2002). However, the presence of circulating DNA in the serum of patients with GBM has heretofore not been assessed.

There is evidence that the blood-brain barrier is broken down in patients with GBM. It has been demonstrated that vasculature in and around gliomas is markedly abnormal with several changes thought to compromise vascular integrity and the blood brain barrier. Changes include breakdowns in gap junctions, basal membrane defects, and fenestrations of vessel walls (Hirano, Kawanami et al. 1994; Machein and Plate 2000; Vajkoczy and Menger 2000) It is plausible that this breakdown in the blood-brain barrier could allow DNA released from the tumor into the serum.

### *Goals*

The goal of this study would be to identify the presence of cell-free DNA in the pre-operative serum of patients with GBM confirmed by surgical pathology compared with leukocyte intracellular DNA of the same patients used as a control. If cell-free DNA is present, we would use DNA amplification/PCR to look for the most common GBM-specific genetic changes in the circulating DNA, such as LOH on 10q and 10p, EGFR amplification, TP53 mutations, p16 deletion and PTEN mutations.

If successful, this study could identify a possible screening test for patients with suspected GBM. In the future this might allow not only serologic diagnosis of GBM, but perhaps other characterization of intracranial neoplasms including factors suggestive of better response to specific/targeted therapies. It could also be studied prospectively for possible use in detecting post-resection tumor recurrence and distinguishing recurrence from post-radiation necrosis.

## **2. Study Design and Statistical Procedures**

We will take 20ml of serum from the Department of Neurological Surgery tumor bank from 30 patients with histologically confirmed GBM. These patients will be identified using the existing Department of Neurological Surgery's database. Once identified each sample will be assigned a sequential unique identifier and any all protected health information (PHI) will be removed from the study records. This selection will be done entirely by the PI and research personnel in the PI's office and associated laboratory.

As a positive control we will isolate intracellular DNA from circulating leukocytes from the same 30 patients. We will also isolate DNA from the tumor tissue specimens to help assess the sensitivity of our assay and discriminate between "false negatives" and "true negatives" of the cell-free serum assay relative to the patients' tumors' genetic characteristics.

Each sample will be analyzed PCR and microsatellite analysis to look for presence of 8 of the most common genetic markers of GBM: LOH at 10q, 10p, 1p and 19q, EGFR

amplification, *PTEN* mutation, *TP53* mutations, and p16<sup>INK4α</sup> deletion. The primary outcome will be positivity on any one of PCR probes, with secondary outcomes of positivity on specific probes. We will analyze the data using chi squared test against the null hypothesis that none of these probes will be positive.

The number of samples needed for this study was determined using a power analysis for a chi-squared test to compare proportions. As this is a novel study we did not have significant evidence in the literature to inform our expectations. We thus decided that a screening test which was less than 30% sensitive would be of little clinical value and performed the calculations to achieve a power of 80%.

### **3. Study Procedures**

No procedures will be performed on patients during this study.

### **4. Study Drugs/Devices**

None.

### **5. Study Questionnaires**

None.

### **6. Study Subjects**

Study group: Blood samples from 30 consecutive patients with radiographically apparent intracranial mass with subsequent biopsy confirmation of histologic diagnosis of glioblastoma multiforme will be identified in the Columbia University Department of Neurological Surgery Tumor Bank. No exclusion criteria will be applied to our study population.

### **7. Recruitment**

Patients will be identified as described above through the database for the Department of Neurological Surgery's tumor bank. The patients have already signed a general consent for their specimens to be a part of the Department of Neurological Surgery Tumor Bank for use in future studies.

### **8. Confidentiality of Study Data**

All data from the study will be kept in the PI's office and associated lab, which are locked and accessible only to lab personnel, the PI, and the PI's staff. As described above, once the serum samples are identified within the tumor bank all PHI will be removed from the study database creating a deidentified database.

### **9. Potential Risks**

The only risk faced by patients in this study is loss of confidentiality.

### **10. Potential Benefits**

The patients whose serum is used will not benefit from the study.

### **11. Alternatives**

N/A

## 12. Location of Study

The study will be performed in the Gabrielle Bartoli Brain Tumor Lab, 5<sup>th</sup> Floor P&S building.

## 13. Compensation

No compensation will be offered patients whose serum is used in this study.

## 14. Costs to subjects

None.

## 15. Minors as Research Subjects

N/A

## 16. Radiation or Radioactive Substances

No radiation or radioactive studies will be used in this study.

## 17. References

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