Dichloroacetate as a Metabolic Treatment for Glioblastoma

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DICHLOROACETATE AS A METABOLIC TREATMENT FOR GLIOBLASTOMA

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of
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Abstract

Glioblastoma is the most common aggressive primary brain tumor in adults, and there is no current standard of care for progression. The Warburg effect is a known part of cancer’s metabolic reprogramming and has been studied as a possible target for limiting tumor growth. Dichloroacetate, a pyruvate dehydrogenase kinase inhibitor, has been proposed as a treatment for glioblastoma, and the drug has had success in phase 1 trials. The proposed study is an open-label, randomized phase 2 clinical trial. Participants will be randomized to oral dichloroacetate, with dosing based on genetic testing, or to a control group treated with lomustine. We propose that treating patients with recurrent glioblastoma with dichloroacetate will result in a statistically significant increase in survival when compared to patients treated with lomustine. In addition, this study will constitute an advance toward the metabolic targeting of the Warburg effect as a treatment for glioblastoma.
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List of Abbreviations

GBM- glioblastoma
DCA- dichloroacetate
PDK- pyruvate dehydrogenase kinase
SOC- standard of care
NDU- newly diagnosed unmethylated disease
NDM- newly diagnosed methylated disease
RD- recurrent disease
OS- overall survival
PFS- progression free survival
GSTZ1- glutathione S-transferase Zeta-1
PDC- pyruvate dehydrogenase complex
CTCAE - Common terminology Criteria for Adverse Events, version 5.0
RANO- response assessment in neuro oncology
SNP- Single Nucleotide Polymorphism
WHO- World Health Organization
IDH- isocitrate dehydrogenase
CHAPTER 1: INTRODUCTION

1.1 Background

Glioblastoma is the most common aggressive primary brain tumor in adults and has an incidence rate of 3.19 per 100,000 persons in the US. It is a major area of research due to its high mortality rate and low survivability, especially at recurrence. Glioblastoma cells appear like glial cells, which support neurons. A distinguishing factor between types of gliomas is the mutational status of isocitrate dehydrogenase (IDH). IDH is an enzyme that participates in metabolism. Glioblastomas are categorized into IDH wildtype, and other gliomas possess IDH mutations. IDH wildtype tumors are typically the most aggressive and associated with a poor prognosis. The standard for glioma nomenclature and diagnosis is the World Health Organization (WHO) classification. In the most recent 2021 WHO Classification, the diagnosis of glioblastoma was restricted only to IDH wild type tumors.

The pathogenesis of glioblastoma is multifactorial and not fully understood. A 2022 review of the literature analyzed many proposed protective factors, risk factors, and genetic mutations that may contribute to the formation of glioblastoma. The review lists 10 genes that indicate prognostic and predictive information, including O-6-methylguanine-DNA methyltransferase (MGMT) methylation status. The MGMT gene has the most impact on clinical practice, and it corresponds to a DNA repair enzyme that repairs DNA to protect cells from carcinogens. However, this mechanism also allows cancer cells to become resistant to alkylating chemotherapy. Thus, patients with methylated tumors have less of the MGMT enzyme and are known to respond better to temozolomide chemotherapy. The gene also corresponds to prognostic data including overall survival. Patients with methylated tumors had
a median overall survival of 21.7 months, while patients with unmethylated had a median overall survival of 12.7 months.\textsuperscript{5,6}

One notable protective factor is female sex and female sex hormones. The incidence in males is higher than that of females, and researchers such as Cowppli-Bony et al found an increased risk of glioma with later menarche and menopause. Additionally, the study demonstrated that exogenous hormones such as hormone replacement therapy and oral contraceptives reduced the risk of glioma.\textsuperscript{7} Other proposed protective factors are the use of NSAIDS, antihistamines, statins, cannabinoids and atopy. However, conclusive evidence to support a clear benefit to these factors has not yet been indicated in the literature. The review also found little evidence to support proposed risk factors such as cigarette smoking, nitrosamines, race, head injury, alcohol use, diet, and obesity. Ionizing radiation was cited as one of the only recognized risk factors with substantial evidence. It was reported to cause a dose proportional increased risk for CNS cancer after radiotherapy to the head as compared to the general population.\textsuperscript{7}

The initial treatment for glioblastoma at diagnosis is separated into three phases. The first phase consists of maximum possible safe resection of the tumor. The second phase of treatment is 6 weeks of chemoradiation with temozolomide, and the final phase of initial treatment is adjuvant monthly temozolomide for 6 to 12 months.\textsuperscript{8} Tumor treating field therapy, a noninvasive treatment which involves transcutaneous delivery of alternating electrical frequencies, has also been approved in newly diagnosed glioblastoma. This therapy has a proposed mechanism to stop tumor growth through magnetic fields triggering a cessation of mitosis.\textsuperscript{9}
At recurrence, treatment is not standardized, though temozolomide rechallenges are often attempted. In recent years, bevacizumab, a monoclonal antibody that binds to VEGF, has been approved. However, despite this new addition and its effects on progression free survival, overall survival has not improved. Participation in clinical trials is often encouraged.

The median survival in newly diagnosed glioblastoma is still only 15 months. As of 2022, 6.9% of patients survive 5 years following the diagnosis. Survival rates have not improved with the addition of targeted therapies. A notable recent large trial included vaccination with rindopepiumut, which eliminates cells that express EGFRvIII\textsuperscript{10}. These cells are present in 30% of primary glioblastoma. Survival statistics are even lower for recurrent glioblastoma, so it is imperative to fill gaps in clinical research. The current standard of care for recurrent glioblastoma is under debate, but lomustine (CCNU) is often used.

Additionally, the financial burden on the healthcare system is significant. Temozolomide, which is the standard of care chemotherapy agent for the treatment of glioblastoma at diagnosis, has cost effectiveness ratios that range from $73,586 to $105,234 per quality adjusted life year, when adjusted to 2013 dollars.\textsuperscript{11} This figure does not take into account the cost of new additions such as bevacizumab, nor does it include costs for surgical resection. In 2004, the total cost per patient including laboratory tests, temozolomide, radiotherapy and imaging was estimated to have a range of $17,023 to 195,773. These amounts do not include cost of surgical resection.\textsuperscript{12} The intervention, dichloroacetate (DCA), has been used to treat mitochondrial disorders and has been used in clinical settings for over 30 years. DCA is a small molecule, and primarily targets mitochondrial metabolism. It is a generic drug and is ubiquitous with a low cost.\textsuperscript{13} Potential addition of DCA to glioblastoma therapy has the capacity to be cost effective and accessible for many patients.
Cancer is known to have several types of metabolic reprogramming that contribute to its growth and aggressiveness, and a part of this reprogramming is the Warburg effect. The Warburg effect has been highly studied for almost 100 years and is defined as tumor’s tendency to convert glucose to lactate in the setting of normal oxygen tension. Many papers have followed this effect, and recently it has been suggested that targeting this pathway would be a helpful way to stop tumor growth in its tracks. There is limited data on clinical attempts via pharmacologic agents, but case reports have described benefits from ketogenic restricted diets, which are proposed to have metabolic effects. There are several proposed agents to target metabolism, and one of the most promising is DCA.

**DCA and dosing**

DCA is a pyruvate dehydrogenase kinase (PDK) inhibitor, and when used on cancer cells, it is hypothesized to shift metabolism from glycolytic pathways to oxidative phosphorylation. This effect has not been extensively clinically attempted, but there have been case reports of DCA and a complete response in different cancers, such as lymphoma and stage 4 colon cancer. In a 2021 review of the clinical attempts of DCA usage in brain tumors, Cook et. al stated, “it readily crosses the blood–brain barrier, making it an excellent candidate for managing patients with brain cancers. In addition, the toxicity from DCA treatment is generally limited to a reversible peripheral neuropathy.” Many in vitro and in vivo studies have investigated this path, and it has been specifically studied in glioblastoma.

In one of the first trials of DCA in glioblastoma in 2015, Chu et al established the dose for DCA as 6.25mg/kg. However, after further investigation through a phase 1 trial by Dunbar et al., it has become clear that there are fast and slow metabolizers of DCA. As explained by...
Shroads et al., DCA is metabolized by the enzyme glutathione transferase zeta 1 (EGT) maleylacetoacetate isomerase (MAAI) which is encoded on the GTZ1/MAAI gene. Thus, as proposed by Shroads et al. and Dunbar et al., three single nucleotide polymorphisms (SNPs) will be identified using DNA sequencing to determine haplotype and therefore achieve correct dosing for all patients. Those who are EGT wildtype carriers are fast metabolizers, and non-carriers are slow metabolizers. Therefore, in the proposed study, the dose for each group will be 12-14mg/kg/12hrs and 6-7mg/kg/12hr, respectively. 20 21

**Measurement of the Effects of DCA**

Measuring the effects of metabolic modulation is not yet standardized. Pyruvate breath tests measuring $^{13}$C labeled products, lactate levels, and different types of imaging modalities have been suggested to measure the in vivo effects of DCA. Breath tests have not proven to be precise, as the hepatic pyruvate dehydrogenase metabolism may have a disproportionate contribution. 20 Currently, the most common standardized imaging modality is a positron emission tomography (PET) scan, which measures glucose uptake via a radioactive analogous form of glucose ($2^{-18}$F-fluoro-2-deoxy-d-glucose). This method can be unreliable as certain organs, including the brain, uptake large amounts of glucose at baseline. Additionally, when we seek to understand the Warburg effect, it is vital to distinguish its catabolism. Specifically, if it is converted to lactate or proceeds to oxidative phosphorylation. A novel form of imaging, deuterium metabolic imaging (DMI), is a good candidate to measure the Warburg effect without this issue. DMI can demonstrate changes in metabolism through monitoring of the glycolytic ratio. The glycolytic ratio is defined as the lactate value over glutamate and glutamine (glx). The
ratio has been studied to evaluate the Warburg effect, where lactate levels show the amount of glycolytic metabolism, and glx represents oxidative metabolism.  

1.2 Statement of the Problem

Glioblastoma nearly always recurs and is quite difficult to treat as recurrent disease. Due to its lethal nature, it is essential to investigate alternative treatments to prolong survivability of glioblastoma. There has been limited progress over the past few years in developing a standard of care for recurrent glioblastoma, and it is imperative to find a new way to target this tumor. Scientists have known that the Warburg effect is an essential part of tumor metabolism for a century, but the idea of targeting these specific pathways has reached a renaissance in the past few years. DCA has been trialed in several types of cancers, and its ability to freely cross the blood brain barrier makes it a good choice for treatment of a primary brain tumor, like glioblastoma. Phase 1 trials by Chu et al. and Dunbar et. al demonstrated recommended phase 2 dosing for DCA in glioblastoma. This proposal describes the next step, moving to a phase 2 trial of DCA in recurrent glioblastoma.

1.3 Goals and Objectives

The broader goal of the study is to investigate the effect of targeting altered metabolism in glioblastoma and attempt to improve treatment for recurrent glioblastoma. Building upon the new field of metabolic treatments, we designed a study to understand if DCA is effective in halting progression of recurrent glioblastoma via metabolic modulation.
1.4 Hypothesis

We hypothesize that administration of DCA twice a day, with dosing based on EGT carrier status (12-14mg/kg/12hr in carriers and 6-7/mg/kg/12hrs in non-carriers) in adult patients, to patients diagnosed with recurrent glioblastoma will improve survival in a statistically significant manner, when compared to patients treated with lomustine 110 mg/kg every six weeks, with a maximum dose of 200mg.

1.5 Definitions

DCA – a pyruvate dehydrogenase kinase inhibitor, the intervention

Lomustine – an alkylating agent, the control

Glioblastoma – aggressive primary brain tumor

Recurrent disease – Glioblastoma that has recurred as evidenced by radiographic or clinical findings.
References


CHAPTER 2: REVIEW OF THE LITERATURE

2.1 Introduction

A full review of the literature was conducted using PubMed, Ovid Medline, SCOPUS, and clinicaltrials.gov. Search terms included a combination of subject headings and key words to describe metabolic reprogramming, overall survival, lomustine, dichloroacetate and glioblastoma. Acronyms and brand names for medication were also used in the search terms. The search strategy was modified for each database and the search continued until the 15th of December 2022. To begin, a broad PubMed search was completed using the filters for “clinical trial, randomized controlled trial, and systematic review” and the terms “DCA and cancer,” which yielded 86 results. In a November 2022 search, entering “dichloroacetate AND glioblastoma” on PubMed yielded 35 results. A similar search on PubMed for “lomustine AND glioblastoma” yielded 359 results. On clinicaltrials.gov, these terms yielded 4 results and 54 results, respectively. A narrowed search on PubMed including “overall survival AND lomustine AND glioblastoma” yielded 257 results. These modified searches on the same date on SCOPUS yielded 38 results, and 82 results on OVID Medline, without any filters on either database.

For searches with many results, papers were included based on year of publication, prioritizing those with more recent dates, completion of the trial if applicable, and overall relevance to the discussion. The review of the literature demonstrates the novelty of the topic, and thus there are minimal studies specific to recurrent glioblastoma and dichloroacetate.

2.2 Review of the Warburg Effect and Dichloroacetate Inhibition

The Warburg effect describes a tumor’s ability to use aerobic glycolysis and forego mitochondrial oxidative phosphorylation despite normal oxygen tension, thus increasing its ability for quick growth. One of the ways in which tumors do this is upregulation of hypoxia
inducible factor to activate pathways used when the cell does not have access to oxygen.

Hypoxia induced factor activates PDK, which in turn inhibits pyruvate dehydrogenase complex (PDC) through phosphorylation of its E1a subunit, inhibiting its catalytic ability. PDC is vital for many reasons, primarily because it is the enzyme involved in the rate limiting step of mitochondrial oxidative phosphorylation. Additionally, PDC aids in the conversion of pyruvate to acetyl-COA and transfer from the cytoplasm to the mitochondria to begin the tricarboxylic acid cycle. Thus, when PDC is inhibited by PDK, it forces the cell to use glycolysis instead. DCA is a known PDK inhibitor, so its use can stop this pathway. When DCA inhibits PDK, it can no longer inhibit PDC, and the cell will use aerobic oxidative phosphorylation once more.  

![Diagram of PDC's conversion of pyruvate and PDK's inhibition by DCA. Panel A: PDC's mechanism of action under normal oxygen tension. Panel B: PDK's inhibition of]  

Figure 2.2 demonstration of PDC’s conversion of pyruvate and PDK’s inhibition by DCA. Panel A: PDC ‘s mechanism of action under normal oxygen tension. Panel B: PDK’s inhibition of
**PDC under normal oxygen tension, caused by tumor cells release of hypoxia induced factor.**

**Panel C: DCA’s inhibition of PDK, thus reversing this effect.**

### 2.4 Review of Evidence for Dichloroacetate in Glioblastoma

Due to the novelty of the subject, the use of DCA as a cancer treatment has not been extensively clinically attempted. In a 2016 in vivo study using glioma C6 cells in Wistar rats, a significant antitumor effect and increased lifespan of rats by 25.5% (p<.05) under prolonged (daily for 13 days) administration of DCA was found. It showed a maximal effect of increase of lifespan by 34.5% (p<.005) at a dose of 1.5g/kg. Other studies in cell lines and rats have also shown this association, including a rat model of glioma that found significant differences in glucose metabolism and apoptosis of glioma cancer stem cells, and a significant increase in overall survival in the mice treated with DCA. Other studies investigating similar cell lines and synergy with other agents have also found statistically significant improvements in overall survival.

Thus, several papers have shown the proposed mechanism in vitro and animal models. As discussed above, additional studies demonstrated DCA’s safety and potential signs of efficacy in humans. Chu et al. completed a phase 1 study of DCA in glioblastoma, using twenty-four patients with solid tumors and found no dose limiting toxicities in the cohort. The starting dose was 6.25mg/kg of DCA administered twice daily and established this as a safe dose and recommend for phase 2 trials of DCA in solid tumors. When this dose was tolerated, patients were escalated to 12.5 mg/kg twice a day. At higher doses, dose limiting toxicities were fatigue, nausea, and neuropathy and adverse events were mainly grades 1-2, with some grade 3 fatigue and neuropathy. No grade 4 events were reported. The study also investigated the
metabolic effects of DCA via PET scans. Of the 24 patients, fourteen were evaluable, and the response was estimated based on the European Organization for research and treatment of cancer (EORTC) guidelines. Of the fourteen patients, seven had stable disease, four had progressive disease, and three demonstrated partial metabolic response. This response was defined as either a >15% decrease after one cycle or a >25% decrease after more than one cycles. Thus, Chu et al. were not able to demonstrate efficacy but found a dose of 6.25mg/kg twice a day recommended for patients in a phase 2 trial.

Another recent study with DCA was Dunbar et al.’s phase 1 trial investigating the safety and tolerability in patients with recurrent glioblastoma or metastases from a primary cancer outside the central nervous system. Interestingly, the study found that genotyping patients was imperative to find the dose limiting toxicity, as there are fast and slow metabolizers of DCA, as discussed above. The trial found that DCA had no statistically significant adverse events. In this study, 15 patients with recurrent malignant brain tumors participated, and all except for one patient stayed on the same dose for the entirety of the study. Patients self-administered oral DCA at 8:00am and 8:00pm, and medication diaries were used to monitor adherence. One patient lowered his dose to 6.25mg/kg due to a grade 1 peripheral neuropathy. The only notable dose limiting toxicities included reversible peripheral neuropathy and reversible asymptomatic elevation of liver enzymes.

The study also used a pyruvate breath test to monitor DCA activity in vivo. During this test, patients fasted overnight and were then administered 25mg of sodium-$^{13}$C pyruvate dissolved in 25 ml tap water. After drinking, patients completed breath test measuring their baseline $^{13}$CO$_2$ production. This was then compared to $^{13}$CO$_2$ production after administration of DCA. A notable product of PDC’s decarboxylation of 1-$^{13}$C-pyruvate is $^{13}$CO$_2$.
breath test can be used to quantify DCA’s action on PDC. Results of the breath test were limited, as only 4 patients were able to complete it. DCA increased the rate or magnitude of $^{13}\text{CO}_2$ production in 2 patients, but the decreased rate was seen in the other 2 patients. Authors of the study concluded this pattern may be due to the breath tests measure of primarily hepatic PDC activity, and it is not yet known if this may be a helpful indication of PDC and DCA’s activity.

Though this trial was not designed to detect drug efficacy, measures of overall survival and DCA efficacy were monitored during the study. The mean survival duration was 140 days from the start of DCA and 88 days from the withdrawal of DCA. During the first 4 weeks of DCA administration, the drug was associated with clinical and radiographic evidence of progression free disease in all eight of the evaluable patients. The study concluded that oral DCA was well-tolerated in patients with recurrent malignant gliomas at the dose range established for metabolic diseases.  

The data on the efficacy of DCA on recurrent glioblastoma is limited, but interesting evidence is reported on 5 patients in the Michelakis et. al study. This study involved both in vitro and in vivo investigation. The in vitro portion of the study focused on the effects of DCA on mitochondria and contained a sample of 49 freshly isolated primary glioblastoma tumors. These tumors were compared to a control of non-cancerous tissue obtained during epilepsy surgery. Then, tissues were treated with DCA or normal saline (vehicle). Thus, the study was able to compare glioblastoma tissue samples treated with DCA, glioblastoma tissue samples treated with normal saline, control tissues treated with normal saline, and control tissues treated with DCA. After injection with a mitochondrial specific dye (mitoSOX), DCA showed mitochondrial depolarization but not in normal brain tissue. Normal saline did not cause
depolarization. This supports DCA’s apparent cancer selectivity and reversible metabolic modulation. Additionally, glioblastoma tissue samples showed a higher concentration of pyruvate dehydrogenase when compared to the control brain tissue. This supports DCA’s proposed mechanism of action, given that PDK is its substrate. Based upon this data, the study moved forward to clinical attempts of DCA in glioblastoma.

These 5 patients were treated with oral DCA for up to 15 months, 3 of whom showed tumor regression on brain imaging and 4 of whom were clinically stable after 15 months of therapy. The patients were administered weight based DCA that was dissolved in water every 12 hours on an empty stomach. The first patient was a 58-year-old man with recurrent glioblastoma after initial surgery, temozolomide, and radiation. The patient had significant regression in two previously PET positive secondary paraventricular masses, and the primary tumor did not increase in size for the 15 months of DCA therapy, 6.25 mg/kg orally twice a day, since month 7. He was not on any other medications during this time. The second patient was a 47-year-old woman who had multiple glioblastoma recurrences and completed debulking surgery followed by chemotherapy 4 months prior to her enrollment. A second debulking surgery and drainage of a symptomatic cyst was performed at month 11. At month 15, she had radiologic improvement and was clinically stable.

**Figure 2.3, Adapted from Michelakis et al, “In vivo effects of DCA”**

Merged PET and T1 gadolinium-enhanced axial MRI images taken before and after 9 and 15 months of DCA monotherapy respectively in patient 1 (left) and patient 2 (right). Note during this time patient two completed two debulking surgeries, while patient 1 did not. The images of patient 1 show apparent regression of the paraventricular mass, while the primary tumor is not visible at the level of this image.
The third patient was a 52-year-old man who had recurrent glioblastoma with an inoperable tumor, significant midline shift, and brain edema. Three months after he began DCA, progressive intracranial hypertension from the mass lead to his death. The fourth patient entered after initial debulking surgery, had evidence of progression after 3 months of DCA monotherapy, and underwent a second debulking surgery. After this surgery, he had nine months DCA in combination with radiation and TMZ followed by 6 months of DCA monotherapy. At the end of the 15 months of this treatment course, he was stable with no radiologic evidence of tumor growth or recurrence. The fifth patient showed complete resolution of the tumor 15 months after enrollment, and her treatment course consisted of TMZ, radiation, and DCA for 6 months, after which she showed progression, and 9 months of DCA monotherapy, during which she showed tumor resolution and remained asymptomatic.

Additionally, following the debulking surgeries and resections, Michelakis et al studied tissue samples from the patients after undergoing DCA therapy. These samples included pre and post therapy tissue samples from patients 2, 3 and 4, and pretherapy tissue samples from patients 1 and 5. The posttherapy samples from all three patients showed fewer cells per unit volume,
decreased proliferation, and increased apoptosis. Levels of proliferating cell nuclear antigen (PCNA)-positive cells and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells were investigated to quantify tumor proliferation and apoptosis, respectively. The number of nuclei was measured to find the decrease in number of cells. These measurements were gathered blindly in eight random fields per slide, with a minimum of three slides per experiment. Recall from section 2.2 that the proposed mechanism for DCA involves the cessation of PDK inhibition of PDC. When examining the post therapy samples from patients 2, 3, and 4, levels of pyruvate dehydrogenase were significantly increased, suggesting that DCA is adequately inhibiting PDK.

Based upon these studies, we propose that DCA will impact metabolically reprogrammed recurrent glioblastoma. There is a need for a phase 2 trial to follow up the success of the phase 1 trials done by Chu et al., Dunbar et al., and Michelakis et al.

2.5 Review of Studies to Assess for Confounders

There are many possible confounders to the primary outcome of overall survival. In a 2021 review, it was noted that an age of <40 years, resection, radiation, and chemotherapy all similarly increased survival time. Confounders for which we will control in the proposed study are those that have an impact on their expected overall survival at baseline. Those who are older, those who have larger tumors (>40mm diameter), and those who have a lower performance status are all expected to survive for a shorter.

To further control for confounding, the proposed study will have stratification factors for randomization. However, given the small sample size, stratification must be limited to one
factor. Assuming it may have the greatest effect on overall survival, this factor will be tumor diameter, which will be dichotomous, >40mm or <40mm.

Other potential confounders are age, Karnofsky performance status, study center and methylation status. Though an important confounder, Karnofsky performance status was not chosen as the stratification factor as it is expected to closely track with tumor size. As previously discussed, it is known that methylation status has an impact on the efficacy of alkylator chemotherapy. It is unknown if this will impact DCA therapy. However, methylation status may impact outcomes in the control arm, since lomustine is an alkylating chemotherapy agent. During the study’s analysis portion, confounding will be controlled using a Cox Proportional Hazard’s multivariate analysis with central review. Variables will include study center to monitor for a cohesive sample.

2.6 Review of Methodology

Study Design, Population and Selection Criteria

When considering the methodology for the study, we closely examined the GBM AGILE study, which was specifically designed as an improved clinical trial for glioblastoma. The study attempts to emulate the ideal trial, with multiple arms in order to minimize redundancy of studies and maximize efficiency. GBM AGILE operationalizes overall survival as the main outcome variable, which we will also use in the proposed study. The use of Bayesian adaptive randomization was helpful in the study due to the differences in the control arms for tumor subtypes (newly diagnosed unmethylated, newly diagnosed methylated, and recurrent disease). However, the proposed study only involves recurrent disease, so it does not need this subtyping.

Randomization is a challenge for many glioblastoma studies. It may be difficult to achieve an adequate sample size at one institution with a double-armed study. One option to
manage these issues is a single armed study, but in a 2022 review of clinical trials and advances in glioblastoma research, Bagley et al highlights the need for a control arm in phase 2 studies. The review critiques the validity of data in single-armed studies and the amount of false positive results. The use of lomustine as a control arm in clinical trials for recurrent glioblastoma has been widely modeled. For example, one of the more recent advances in treatment options for recurrent glioblastoma has been the addition of bevacizumab. The study that demonstrated this finding used a control arm of lomustine alone compared to lomustine and bevacizumab, with the same dosing and formulation as the proposed study.

In order to manage sample size feasibility and avoid undertreatment of patients, the proposed study will use 1:1 randomization. The proposed study also combats sample size and randomization challenges by using informed consent and a lack of blinding, so patients are aware of which treatment they are receiving, and a multi-institutional design to have enough participants.

The Bagley et al. review also cites selection criteria as an area of improvement for glioblastoma trials. Many trials have been critiqued for highly specific inclusion and exclusion criteria that consequently limits the number of patients eligible for the study. The limited eligibility hinders patients from receiving possibly efficacious treatment and reduces the generalizability of results to all patients with glioblastoma. Some principal factors specifically cited in the review are initial temozolomide therapy and radiation, number of progressions, and therapy for prior progressions. The proposed study is specific to recurrent glioblastoma, so patients must be considered treatment failures to be eligible. However, due to these concerns and the frequency of patients with unmethylated disease who do not receive temozolomide at initial diagnosis, the study will allow patients presenting with recurrence after any combination of...
initial therapy that includes a complete course of radiation. Additionally, the study will allow any number of prior progressions and any types of therapy for these progressions. Detailed inclusion and exclusion criteria, adapted from the Dunbar et al. trial, is available in chapter 3 for reference.

**Outcomes, Operationalization, and Statistical Analysis**

The GBM Agile study, Wick et al., and Lombardi et al. use overall survival as the primary endpoint, which is standard for many glioblastoma phase 2 trials. Typical secondary endpoints include progression free survival, response assessment as defined by the response assessment in neuro-oncology (RANO) criteria, and adverse events, which will also be included in the proposed study. The RANO criteria is widely used in glioblastoma studies and is an objective way to evaluate response, progression free survival, and overall survival in glioblastoma patients. As modeled in the above studies, local investigators will evaluate imaging based on this objective method for standardization. A full table of each outcome variable and its operationalization is available for reference in chapter 3.

To analyze these outcomes, the Kaplan Meier method will be used to generate survival curves for the control and treatment groups, and a hazard ratio will be calculated to determine treatment efficacy, using a 95% confidence interval. To assess for confounding and provide a multi-variate analysis, Cox Proportional Hazards analyses will be performed using the previously listed factors. Fisher’s exact test will be used to compare responses, as defined by RANO criteria between the treatment groups. To assess safety in the trial, adverse events as defined by the Common Terminology Criteria for Adverse Events, version 5.0 (CTCAE 5.0) will be compared using a chi-square analysis.
2.7 Conclusion

After extensive review of similar phase 2 clinical trials, we arrived at a plan to proceed with the proposed study design. Many glioblastoma studies, including GBM Agile, which was designed to improve clinical trials in glioblastoma, use a similar design with the same control arm, primary endpoint, and statistical analyses.
REFERENCES


Chapter 3: Study Methods

3.1 Study Design

The proposed study is a multi-institution, randomized, open-label clinical trial to assess the impact of DCA on glioblastoma compared to lomustine. Participants will be randomized to lomustine 110 mg/kg or DCA and genotyped to determine EGT carrier status. EGT carriers will receive a dose of 12-14mg/kg/12hr and non-carriers will receive a dose of 6-7mg/kg/12hr. See the figure below for a detailed review of the study design.

![Study design figure]

*Figure 3.1: Study Design and Timeline*
3.2 Study Population and Sampling

The source population for the study is adults with recurrent glioblastoma, and participants will be recruited from many institutions to ensure an adequate sample size, including Yale’s Cancer Center as the lead center. Participating centers will also include Dana Farber Cancer Institute, Memorial Sloan Kettering Cancer Center, Massachusetts General Hospital, Mayo Clinic, and MD Anderson Cancer Center. In order to be considered eligible, participants must have been previously diagnosed with glioblastoma and have evidence of tumor recurrence as confirmed by surgery or clinical and radiographic assessments.Patients with both methylated and unmethylated glioblastoma will be eligible for the study. Patients who have received treatment and/or surgery for recurrence may be included in the study, provided they have progressed on and fully recovered from the prior treatments.

Inclusion and exclusion criteria are listed in the table below:

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study subjects will be adults, aged 18 through 80 years, including males and females.</td>
<td>Patients considered pre-terminal (life expectancy ≤ 2 months) and patients who are pregnant</td>
</tr>
<tr>
<td>All subjects will have completed initial therapy with some combination including but not limited to surgical debulking, radiation, and temozolomide (TMZ) and will, therefore, be considered treatment failures. Any number of prior treatment failures will be accepted, so long as participants have fully recovered from any prior therapy</td>
<td>Patients with NCI-CTCAE grade 3 peripheral neuropathy (PN) on exam, except when it was considered attributable to the location of the tumor and its prior therapies, due to DCA’s tendency to cause reversible peripheral neuropathy</td>
</tr>
</tbody>
</table>
Subjects must have been previously diagnosed with glioblastoma and have experienced tumor recurrence as determined by neuroimaging and some degree of symptomatology (e.g., headache, mental status change, seizure) | Patients with end stage renal failure (GFR ≤ 30 ml/min); due to DCA’s reduced clearance in these patients and patients with severe liver insufficiency (total bilirubin > 2.0 mg/dl or ALT or AST > 3 x ULN) will be excluded due to DCA’s hepatic metabolism.

Patients with the capacity for childbearing must agree to use a reliable form of contraception (intrauterine device, oral contraceptive, double barrier method) due to the risk of birth defects during treatment with lomustine | Patients who are taking an insulin or a sulfonylurea, due to the possibility of symptomatic hypoglycemia. (DCA inhibits gluconeogenesis and lowers blood glucose in patients with type 2 diabetes)

Patient must have a Karnosky performance status of >60% within 14 days prior to randomization to be considered eligible. | QTc > 450 msec if male and QTc > 470 msec if female

Adequate bone marrow function: hemoglobin >9.0 g/dL, white blood cells >3·0 × 10⁹/L, absolute neutrophil count >1500 per mm³ without transfusion or granulocyte colony stimulating factor, platelet count ≥100 000 per µL, due to bone marrow suppression during treatment with lomustine | Patients with known human immunodeficiency virus, hepatitis B or C, will be excluded from the study. Lomustine makes patients vulnerable to opportunistic infection via bone marrow suppression, so patients with HIV would be further at risk.

Patients receiving active treatment for GBM outside of the trial will not be eligible. | History of interstitial lung disease or symptoms of pulmonary illness, cough, dyspnea.

*Table 3.2: Inclusion and Exclusion criteria*
Sample Size Calculation

Sample size calculations were completed using power and precision (Englewood, New Jersey). For the control arm of lomustine, the incidence rate of 75%, with 329 overall survival events, was used from the data in the Wick et al. study. In order to calculate effect size, hazard ratios were conducted via the Michelakis et al. paper, during which 5 patients were followed for 15 months, with 1 survival event. However, due to the small sample size of the study, effect size is most likely overestimated at 20% incidence. Thus, the effect size was reduced in calculations, as shown below. The study uses a power of 0.81 and a two-tailed alpha of 0.05. This yields 31 persons in each group, with a total of 62 research participants. See appendix A for more details.

3.3 Subject Protection and Confidentiality:

Each participant will receive an informed written Consent Form that is compliant with each center’s institutional review board before any study procedures are initiated. When the participant is given the form, one of the investigators will explain it in detail and answer any concerns. If necessary, interpretation services specific to each center will be used, and interpreter information and ID will be recorded. The informed consent will delineate specific details, such as the ability to withdrawal from the study at any time for any reason. Additionally, if any new treatment information becomes available during the study, the participants will be informed, as it may affect their willingness to participate in the study. The Consent Form will describe the intervention, time course of the study, and clinical procedures to be completed. A sample Informed Consent Form is provided in the appendix. Participants will be given ample time to review the Consent Form and discuss with family members prior to giving their consent. Confidentiality of the subjects will be assured by assigning each subject a participant ID that is
unique and non-identifiable. Compliance with study protocol will be enforced with current Health Insurance Portability and Accountability Act (HIPAA) regulations through oversight of the principal investigator. The study will be publicly registered on clinicaltrials.gov.

3.4 Recruitment

Multiple institutions will be included to obtain an adequate sample size. Subjects will be recruited from many cancer centers in the US. These centers will include Yale Cancer Center, Dana Farber Cancer Institute, Memorial Sloan Kettering Cancer Center, Massachusetts General Hospital, Mayo Clinic, and MD Anderson Cancer Center. The study will be offered to eligible participants during treatment discussions after presenting with recurrent glioblastoma.

3.5 Study Variables and Measures:

DCA will be self-administered by patients twice a day at 8:00am and 8:00pm. Those participants randomized to the DCA arm will be genotyped to determine EGT carrier type status. Carriers will receive 12-14mg/kg/12hrs, and non-carriers will receive 6-7mg/kg/12hrs. The formulation for DCA is a liquid combined with strawberry flavoring and aspartame, which was used in the phase 1 trial. Participants in this group will be compared to those who receive lomustine 110mg/kg every 6 weeks. Lomustine is an alkylating agent widely used in clinical trials as a comparator. Those in the lomustine group will self-administer a capsule at bedtime every six weeks. Blinding of the intervention is unnecessary and non-standard at this stage, and we don’t believe it would aid in the outcome measurement of overall survival.

The primary outcome for the proposed study is overall survival, which is defined as date of randomization until death. Data from patients who withdraw from the study or are lost to
follow up will be censored from the last date of evaluation. Patients who are still alive will also have data censored at last date of evaluation. Secondary end points will include progression free survival, response rates as determined by the RANO criteria, and adverse events. Progression free survival is defined as the time from enrollment to any progression event, as defined by the RANO criteria. Operationalization of these variables are in the table below.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Operationalization</th>
<th>Type of Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Survival (12 months)</td>
<td>Time to event</td>
<td>Kaplan Meier</td>
</tr>
<tr>
<td>Progression Free Survival</td>
<td>Time to event</td>
<td>Kaplan Meier</td>
</tr>
<tr>
<td>Response Assessment</td>
<td>Categorical</td>
<td>Fisher’s Exact</td>
</tr>
<tr>
<td>Adverse Events</td>
<td>Ordinal</td>
<td>Chi Square Analysis</td>
</tr>
</tbody>
</table>

Table 3.4: Operationalization of key variables in the proposed study.

3.6 Assignment of Intervention, Adherence, and Adverse Events

Subjects will be randomized using simple randomization with stratification for tumor size, <40mm or >40mm. The randomization will be completed via a web-based system in a 1:1 ratio. Adherence will be monitored using medication diaries, medication levels when labs are drawn, and return of unused medication. Adverse events will be monitored using case reports and the CTCAE version 5.0. As previously stated, the most common reported adverse event with DCA therapy is peripheral neuropathy. This side effect will be specifically monitored at clinical checkups via patient history and monofilament testing. Neurologist at the sites will be trained to use a 5.07/10g monofilament at several locations on the feet and hands. This testing will be standardized throughout the study, and a sample training pamphlet is in Appendix C.
3.7 Data Collection

At baseline, patients will receive a full clinical evaluation, including neurological exam and imaging. Imaging will include MRI studies to assess tumor status based on RANO criteria. Patients who have had an MRI in the last 30 days will not be required to repeat imaging. Laboratory assessments will include complete blood count, complete metabolic panel, and urinalysis.

Following the baseline evaluation, participants will be monitored with return visits every 2 weeks, with clinical assessments including vital signs, complete blood counts, blood chemistries, adverse event monitoring, and urinalysis. After 8 weeks into the study, participants will begin a pattern of clinical assessment with vital signs, complete blood counts, blood chemistries, adverse event monitoring, urinalysis, and MRI brain with contrast every 2 months. The imaging will be assessed by two independent reviewers using the RANO criteria.

3.8 Statistical Analysis

Data from the study will be analyzed by a statistician independent of the study, and overall survival and progression free survival will be tested using Kaplan Meier curves, which will be conducted in the intention to treat population. Patients who are still alive will be censored at the last date of follow up, and patients who are lost to follow up will also be censored on the last date of evaluation. The treatment group will be compared to the control group using a log rank test. Cox Proportional Hazards method will also be used to perform a multivariate analysis to account for confounders. Factors include: KPS score, largest diameter of tumor <40mm or >40mm, methylation status, and age.
3.9 Timeline and Resources

The study will take place over 2 years. The recruitment period will be 1 year, and multiple sites will be activated to allow for sufficient recruitment of eligible participants.

The resources needed for the study include clinic space, which will be provided by the study centers, and DCA and lomustine, which will be acquired through the site’s pharmacy after approval from the Investigation Drug Service (IDS). Yale’s Cancer Center will be the lead study site, and as such the national principal investigator will be Yale faculty. Each site will require a site principal investigator, which the national principal investigator will be responsible for choosing, and radiology resources to complete periodical evaluations of the participants. To complete the baseline and return evaluations, the study will also require laboratory supplies for blood tests, equipment to monitor vital signs (scale, blood pressure cuff, and a thermometer), and appropriate personnel (RN, LPN, MA) to complete these tests. The study sites will sign a delegation of responsibility document to ensure all research personnel are aware of responsibilities and requirements for the study. A data safety monitoring board will be created to centrally evaluate data from each clinical evaluation and adverse event report to maintain the safety of the study.

During the analysis phase, a statistician independent of the study will perform the analysis of all data. A radiologist will also be necessary to evaluate changes on neuroimaging of the tumor based on the RANO criteria.
CHAPTER 4: CONCLUSION

4.1 Advantages and Disadvantages

A key advantage to the study is its external validity and thus its generalizability to patients with glioblastoma. As discussed in chapters 2 and 3, inclusion criteria were broadened as much as possible to include glioblastoma patients who had received different types of therapy. The study design is reasonable and established, given the number of prior glioblastoma studies’ use of the same control arm and primary endpoint.

A potential limitation to the study is the control arm of lomustine. Though its use as a comparator is common, it has many toxic side effects, and there is no standard of care in recurrent glioblastoma. Lomustine is a potent bone marrow suppressant and can leave patients fatigued and vulnerable to opportunistic infections. It is difficult to design a trial comparing a new treatment to conventional treatments, as there are few options for patients diagnosed with recurrent glioblastoma. Another possible limitation of the study is calculation of effect size. Though the sample size is average for a phase 2 trial, the novelty of DCA makes it difficult to accurately predict effect size, and the relative scarcity of patients and subspecialty clinics limits the scalability of interventions. If the study is successful, a larger, phase 3 trial will be indicated.

4.2 Clinical Significance

Over the past 10 years, much research has resulted in improved outcomes in different types of cancers via targeting specific pathways. Notable examples include the development of tyrosine kinase inhibitors and all-trans retinoic acid for acute promyelocytic leukemia. The Warburg effect has been extensively studied for a century; however, it has not yet been successfully targeted. Metabolic treatments are novel and may demonstrate a useful targeted approach in glioblastoma therapy. If successful, the study has the potential to demonstrate
effective modulation of known metabolic derangements in cancer to cause downstream clinical effects. However, potential implications of the study findings are not limited to a successful demonstration of increased overall survival. The preclinical development of metabolic imaging with DMI may provide a direct measure for the mechanism of action of DCA, providing another biomarker of metabolic therapy in clinical trials. Future directions include deploying DMI as a novel means to understand more about the Warburg effect and its significance. For instance, if a study were to find an impact on the Warburg effect but no corresponding impact on tumor growth or survival, it would lead us to believe that the Warburg effect may not be a crucial target for future therapies. Conversely, if the Warburg effect was unchanged on imaging, we would learn that DCA may not be a proper target for its inhibition. Finally, if we were to see an impact on both the Warburg effect and overall survival, it would provide additional evidence of DCA as an effective treatment in glioblastoma.

As previously discussed, glioblastoma has a high mortality rate and a low survivability.\textsuperscript{1} Treatments are currently limited, and they rely heavily on tumor resection and alkylating chemotherapies such as temozolomide. Additionally, the burden on the health care system is significant, as is the social burden for patients and families when receiving this diagnosis. Research in this field is constantly ongoing and it is imperative to explore every avenue to improve these issues. Thus, though it is our hope the trial demonstrates an increase in overall survival and decreased burden of disease in patients with glioblastoma, if it is not successful, we will have achieved further in-depth exploration of the concept of metabolic treatment of cancer. We posit that any attempt to further the treatments for glioblastoma is worthwhile. As medicine continues to advance understanding the mechanisms of specific pathways will lead to continued
progress. We are hopeful that we will succeed in our goal of increasing survival time and improving therapy for these patients with continued efforts and research.
References

Appendices

APPENDIX A: SAMPLE SIZE CALCULATION

The sample size was calculated with the following:
Alpha: 0.05 (tails = 2)
Beta: 0.20 Power of 81%
Effect Size for proportions: 35, yielding the final sample size of 62 subjects
APPENDIX B: SAMPLE INFORMED CONSENT FORM

CONSENT FOR PARTICIPATION IN A RESEARCH PROJECT

200 FR. 1 (2016-2)
YALE UNIVERSITY SCHOOL OF MEDICINE – YALE-NEW HAVEN HOSPITAL

Study Title: DICHLOROACETATE AS A METABOLIC TREATMENT FOR GLIOBLASTOMA
Principal Investigator: [Insert name.]

Invitation to Participate and Description of Project

You are invited to participate in a research study designed to investigate the effects of the medication dichloroacetate as a potential treatment for glioblastoma. You have been asked to participate because you have been diagnosed with recurrent glioblastoma. There will be 62 research subjects, and seven sites. Each subject will be randomized to receive either dichloroacetate or lomustine (CCNU), which is routinely used in the care of recurrent glioblastoma. In order to decide whether or not you wish to be a part of this research study, you should know enough about its risks and benefits to make an informed decision. This consent form gives you detailed information about the research study, which a member of the research team will discuss with you. This discussion should go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures, possible benefits, and possible alternative treatments. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

Description of Procedures

Each participant will be randomized to either dichloroacetate or lomustine (CCNU) using a web-based randomization system (computer generated randomization). Then, each participant will complete a baseline medical evaluation before initiation of treatment. The baseline medical examination will include:
- MRI, which is part of your routine clinical care.
- Blood draw for routine laboratory test (complete blood count, comprehensive metabolic panel)
  This will require only a few teaspoons of blood.

After the initial evaluation participants will be required to come to clinic for evaluation every 2 weeks, then after 8 weeks, visits will be every 2 months thereafter.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time. You will be told of any significant new findings that are developed during the course of your participation in this study that may affect your willingness to continue to participate.

Risks and Inconveniences
Dichloroacetate is not an approved drug for treatment of any condition, and this intervention is still investigational. Participation in this study may involve risks that are currently not known. Most subjects in a similar study had peripheral neuropathy, nausea, and fatigue.

There is a federal law called the Genetic Information Nondiscrimination Act (GINA) that, in general, makes it illegal for health insurance companies, group health plans, and most employers, except those with fewer than 15 employees, to discriminate against you based on your genetic information. However, it does not protect you against discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

**Benefits**
Benefits from the study may include improved health outcomes, a better understanding of the glioblastoma that may lead to new treatments, or general advancement of scientific knowledge. Please note that, by definition, the benefits of research are unproven.

**Treatment Alternatives/Alternatives**
You are not required to participate in the study, and there are other options for treatment if you wish to decline. You may choose other strategies such as temozolomide re-trial or palliative care, which helps with symptom management.

**Confidentiality**
Information about your study participation will be entered into your Electronic Medical Record (EMR). Once placed in your EMR, these results are accessible to all of your providers who participate in the EMR system. Information within your EMR may also be shared with others who are appropriate to have access to your EMR (e.g. health insurance company, disability provider.) Authorized representatives of the Food and Drug Administration (FDA) and the manufacturer of dichloroacetate may need to review records of individual subjects. As a result, they may see your name; but they are bound by rules of confidentiality not to reveal your identity to others.

Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. Data will be coded with numbers and stored on a password-protected computer. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity unless your specific consent for this activity is obtained.

Representatives from the Yale Human Research Protection Program, the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects) may inspect study records during internal auditing procedures. However, these individuals are required to keep all information confidential.

**In Case of Injury**
If you are injured while on study, seek treatment and contact the study doctor as soon as you are able.
Yale University and Yale-New Haven Hospital do not provide funds for the treatment of research-related injury. If you are injured as a result of your participation in this study, treatment will be provided. You or your insurance carrier will be expected to pay the costs of this treatment. No additional financial compensation for injury or lost wages is available.

You do not give up any of your legal rights by signing this form.

**Voluntary Participation and Withdrawal**
Participating in this study is voluntary. You are free to choose not to take part in this study. Refusing to participate will involve no penalty or loss of benefits to which you are otherwise entitled (such as your health care outside the study, the payment for your health care, and your health care benefits). However, you will not be able to enroll in this research study and will not receive study procedures as a study participant if you do not allow use of your information as part of this study.

**Withdrawing From the Study**
If you do become a subject, you are free to stop and withdraw from this study at any time during its course.

To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part. This will cancel any future appointments. The researchers may withdraw you from participating in the research if necessary, such as instances with progression of disease, development of serious side effects, or non-adherence to medication.

Withdrawing from the study will involve no penalty or loss of benefits to which you are otherwise entitled. It will not harm your relationship with your own doctors or with Yale-New Haven Hospital, or any other study center. We would still treat you with standard therapy or, at your request, refer you to a clinic or doctor who can offer this treatment.

When you withdraw from the study, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to ensure the integrity of the study and/or study oversight.

**Questions**
We have used some technical terms in this form. Please feel free to ask about anything you don't understand and to consider this research and the consent form carefully – as long as you feel is necessary – before you make a decision.

**Optional Specimens for Future Storage/Genetic Testing**
You are invited to allow some of your samples (called specimens) and related information to be stored (banked) for future research in cancer therapy. This may help researchers in the future learn more about how to prevent, find and treat Glioblastoma.

Your specimens will be stored for an unlimited time and may be used to make a cell line that will live indefinitely. Future research may look at your genes, which are the units of inheritance that are passed down from generation to generation. Genes are responsible for many things about you such as eye color, hair color, blood type and hundreds of other traits. Future genetic analysis may possibly include finding out the details of how your DNA is put together, such as whole exome or genome sequencing, or genome wide association studies (that is, looking at genes other than those associated with a specific disease). The materials at some point may be injected into animals in some of the research. We expect that there will be widespread sharing of these specimens and associated information.

When your specimens and information are stored, we are careful to try to protect your identity from discovery by others. Your samples and information will receive a unique code. Other researchers will only receive coded samples and information and will not be able to link the code to you. Strict security safeguards are in place to reduce the chance of misuse or unplanned release of information.

Using your specimens for research will probably not help you. We do hope the research results will help people in the future.

There is a risk that your information could be misused. The chance of this happening is very small. We have protections in place to lower this risk, such as protecting samples via unique code. There can also be a risk in uncovering genetic information. New health information about inherited traits that might affect you or your blood relatives could be found during a research study. Very rarely, health or genetic information could be misused by employers, health insurance companies, and others. There is a federal law called the Genetic Information Nondiscrimination Act (GINA) that, in general, makes it illegal for health insurance companies, group health plans, and most employers (except those with fewer than 15 employees) to discriminate against you based on your genetic information. However, it does not protect you against discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Your specimens and information will only be used for research and will not be sold. There is a possibility that this research may lead to development of products that will be commercialized. If this happens, there is no plan to share any financial gain with you.

Research results will not be returned to you or your doctor. If research results are published, your name and other personal information will not be given.
Authorization

I have read (or someone has read to me) this form and have decided to participate in the project described above. Its general purposes, the particulars of my involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Name of Subject: ______________________________

Signature: ____________________________________

Relationship: _________________________________

Date: _________________________________________

Signature of Principal Investigator Date __________________________

or

Signature of Person Obtaining Consent Date __________________________

If you have further questions about this project or if you have a research-related problem, you may contact the Principal Investigator. If, after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at 203-432-5919. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.
APPENDIX C: MONOFILAMENT TRAINING
Adapted from the health resources and services guidelines

1. Show the monofilament to the patient and touch it to his/her hand or arm so that he/she knows it does not hurt.

2. Use the 10 gram monofilament to test sensation at the indicated sites on each foot as shown. Apply the monofilament along the perimeter of and NOT on an ulcer, callous, scar, or necrotic tissue.

3. Hold the filament perpendicular to the skin and use a smooth motion when testing. Use a 3 step sequence that includes (1) touch the skin, (2) bend the filament, and (3) lift from the skin.

Do not use rapid movement. The approach, skin contact, and departure of the monofilament should be approximately 1½ seconds duration. (See Figures 1-3).
4. Ask the patient to respond "yes" when the monofilament is felt. If the patient does not respond when you touch a given point on the foot, continue on to another site. When you have completed the sequence, REPEAT the area(s) where the patient did not indicate feeling the monofilament.

5. Use the monofilament in a random sequence.

6. On the form, indicate with a minus sign, "—", the areas where the patient did not respond to the filament. **Loss of protective sensation at any one of the eight sites indicates a high risk.**


15. Garon EB, Christofk HR, Hosmer W, et al. Dichloroacetate should be considered with platinum-based chemotherapy in hypoxic tumors rather than as a single agent in advanced non-


